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— 协办单位 —

哈尔滨医科大学附属第二医院

目 录

一、基于干细胞的疾病模型

Hsp90 通过调节糖酵解酶复合物在胞浆中的区域化分布促进胃癌的转移和干性

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【摘要】Heat-shock protein 90 (Hsp90) plays a crucial role in tumorigenesis and tumorprogression; however, its mechanism of action in gastric cancer (GC) remains unclear. Here, the role of Hsp90 in GC metabolism is the focus of our research. High expression of Hsp90 in GC tissues can interact with glycolysis, collectively affecting prognosis in clinical samples. Both in vitro and in vivo experiments demonstrate that Hsp90 is able to regulate the migration and stemness properties of GC cells. Metabolic phenotype analyses indicate that Hsp90 influences glycolytic metabolism. Mechanistically, Hsp90 interacts with glycolysis-related enzymes, forming multienzyme complexes to enhance glycolysis efficiency and yield. Additionally, Hsp90 binds to cytoskeleton-related proteins, regulating the regional distribution of glycolytic enzymes at the cell margin and lamellar pseudopods. This effect could lead to a local increase in efficient energy supply from glycolysis, further promoting epithelial–mesenchymal transition (EMT) and metastasis. In summary, Hsp90, through its interaction with metabolic enzymes related to glycolysis, forms multienzyme complexes and regulates regional distribution of glycolysis by dynamic cytoskeletal adjustments, thereby promoting the migration and stemness of GC cells. These conclusions also support the potential for a combined targeted approach involving Hsp90, glycolysis, and the cytoskeleton in clinical therapy.

【关键字】Hsp90, glycolysis, multi-enzyme complex, regionalized distribution, stemness, combination therapy

人的视网膜类器官模型研究 WNT-FZD5 通路对神经祖细胞的分化调控机制

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【摘要】Coloboma represents a spectrum of ocular malformations that account for an estimated 10-15 percent of pediatric blindness. The combined prevalence of microphthalmia and coloboma is ~1:4000. Previously, we found that mutations of Fzd5/FZD5 underlie microphthalmia and coloboma in both mice and humans, characterized by disrupted retinal progenitor homeostasis. To further understand detailed mechanisms of how Wnt signaling contributes to retinal progenitor homeostasis specifically in the human retina, we used ES and iPSC-derived retinal organoids to engineer FZD5 loss-of-function and coloboma disease alleles with the dCas9 nuclease. We observed local rearrangement of the retinal neuroblast polarity in line with these observations in the Fzd5 mutant mice. Single-cell RNAseq analysis revealed abnormal progenitor differentiation and disproportional retinal neuron generation, with an altered cell cycle process. Accordingly, we found increased early born neurons including retinal ganglion cell (RGC) and photoreceptor (PR) in the mutant organoids. Altogether, the data laid a foundation for further investigation of the role of WNT-FZD5 in human retina development and related diseases.

Blockage of C3aR alleviates Parkinsonism phenotype by regulating immune functions in the choroid plexus

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【摘要】Emerging evidence suggests that inflammation, which is associated with immune imbalance, may persist in the pathology progression of Parkinson’s disease (PD). Over the past decade, studies have identified specialized brain niches involved in immune responses, including the meninges, the choroid plexus (CP), and the perivascular spaces. Among them, the epithelial cells of CP form the blood-cerebrospinal fluid barrier (BCSFB) which acts as a selective gate for leukocyte recruitment during inflammation. Recent single-cell profiling has shown that CP harbors a diversity of immune cells, and increased inflammatory signals and immune activity are found in aged CP tissue. It's not fully understood how CP integrates inflammatory signals and mediates immune activities during the progression of PD. In a transgenic mice model of PD, we observed leukocyte infiltration, especially C3aR immune positive monocytes/macrophages, in CP. Furthermore, in a rotenone-induced PD model, we demonstrate an increasing number of C3aR+ monocytes/macrophages recruited to CP through flow cytometry, along with disruption of BCSFB shown by immunofluorescence staining of tight junction proteins. C3aR deficiency (either through administration of a C3aR inhibitor or knockout of the C3aR gene) restored motor function, rescued the aggravated dopaminergic neuron death, reduced gliosis, and decreased leukocyte recruitment to CP in rotenone-induced parkinsonism mice. We also confirmed the migratory capability of C3aR+ cells by labeling peripheral blood. Under an inflammation state triggered by the administration of lipopolysaccharide (LPS), peripheral C3aR+ monocytes can be recruited to CP, leading to inflammatory cytokines release locally. Additionally, we found that activation of C3aR results in polarization of BV2 cells in vitro under inflammatory conditions. Therefore, we provide a new perspective on the role of C3aR controlling CP to moderate brain immunity, which may be considered an effective therapy for the treatment of PD.

【关键字】Parkinson’s disease, C3aR, choroid plexus, leukocyte recruitment

粘附型 GPCR 分子 ADGRE2 通过 PLC-β/PKC/MEK/ERK 信号轴维持 AML 干细胞蛋白内稳态促进 AML 进展

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【摘要】急性髓系白血病（AML）是一种强侵袭性、高异质性的血液恶性肿瘤。在老年患者中，AML 的发病率较高。AML 的治疗目前主流上还是以诱导化疗（“7+3”方案）为主，但是由于肿瘤干细胞（LSC）的存在，极大比例的 AML 患者依然会复发。G 蛋白偶联受体（GPCR）在生理过程和人类疾病中发挥着不可或缺的作用。为了研究 GPCR 在 AML 尤其是 AML 干细胞中的功能，本研究通过生物信息学分析和细胞功能研究对所有>800 个 GPCR 分子在 AML 中的影响进行系统性筛选，鉴定了一个粘附型 GPCR（GPCR 的第二大类别，研究显示近三分之一的粘附型 GPCR 成员在造血干、祖细胞、免疫细胞中表达）成员 ADGRE2 能够非常显著地促进 AML 的功能。我们发现 ADGRE2 在 AML 细胞，尤其是在 LSC 中高表达，并且能够作为 AML 患者预后不良的独立危险因素。在 AML 细胞系以及 AML 患者来源的细胞中沉默 ADGRE2 能够抑制细胞的增殖和克隆形成，促进其凋亡和向下游分化。重要的是，沉默 ADGRE2 能够显著地抑制 LSC 的比例，并且抑制 LSC 的自我更新能力。小鼠体内异种移植实验也显示，抑制 ADGRE2 能极大地延缓 AML 的进展。机制上，我们发现 ADGRE2 能激活磷脂酶 C-β/PKC/MEK/ERK 通路，增强转录复合物 AP1 的表达，AP1 能够驱动蛋白磷酸酶 DUSP1 的表达。DUSP1 可以维持共伴侣蛋白 DNAJB1 的 Ser16 低磷酸化状态，这有助于 DNAJB1-HSP70 相互作用，从而维持 AML 细胞的蛋白内稳态。最后，我们发现联合抑制 MEK、AP1 和 DUSP1 在 AML 异种移植小鼠模型中显示出强大的治疗效果。总之，本研究阐明了 ADGRE2 在 AML 中的作用和机制，并为治疗 AML 提供了一种有前景的治疗策略。

【关键字】急性髓系白血病；G 蛋白偶联受体；肿瘤干细胞；小分子抑制剂；DUSP1

转录因子 X 诱导阿尔兹海默病小鼠模型中反应性星形胶质细胞向功能性神经元的转分化

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【摘要】阿尔兹海默病作为世界第一大神经退行性疾病，临床表现为记忆力和认知能力的渐进性减退，病理特征为细胞外 Aβ沉积、细胞内神经纤维异常缠结，神经元大量丢失同时伴随广泛的胶质细胞激活和神经炎症。目前其具体发病机制仍不完全清楚，尚无有效治疗手段。利用大脑中被激活的星形胶质细胞原位转分化为功能性神经元可以进行细胞替代，进而达到神经修复的目的。然而，目前对星形胶质细胞命运调控机制缺乏足够的了解和直接的证据，致使关于在体情况下再生的神经细胞是否源自星形胶质细胞存在较大的争议性，影响了该领域研究成果的可靠性及临床应用。本项目拟以阿尔兹海默病 5xFAD 转基因小鼠模型为对象，将转录因子 X 特异性的递送至大脑的激活态星形胶质细胞中，通过病毒注射后不同时间点免疫染色、Smartseq2、RNAseq、小鼠行为学检测、神经电生理评估等手段，探索 X 作用下星形胶质细胞的转变过程和转变结果，最终明确证明星形胶质细胞可以体内重编程而产生神经元是否为真实发生的事件。

建立纯合子小鼠地中海贫血的胚胎干细胞系

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【摘要】地中海贫血的治疗性研究离不开细胞模型和动物模型。β^{654} 地中海贫血小鼠只有杂合子能够存活，纯合子在胚胎期即致死。此研究旨在获得纯合子的β654 小鼠的胚胎干细胞（ESCs）以用于作为地中海贫血治疗的细胞模型。杂合子β^{654} 公鼠和杂合子β^{654} 母鼠进行交配后，取 E3.5 囊胚，接种在滋养层细胞上，用 20%SR-Konck DMEM 的多能干细胞培养基进行 5-6 天培养。待胚胎干细胞克隆形成后消化成细胞小团块，用 20%FBS-DMEM 的多能干细胞培养基培养一天后，换成 20%SR-Konck DMEM 的多能干细胞培养基，继续培养 3-4 天，而后进行克隆扩增。对克隆细胞进行 DNA-PCR 鉴定，检测结果有人源β珠蛋白基因片段但无小鼠β珠蛋白基因片段判断为纯合子β^{654}ES 细胞系，有人源β珠蛋白基因片段又有小鼠β珠蛋白基因片段判断为杂合子β^{654}ESCs。通过鉴定，获得了纯合子的β^{654} ESCs、杂合子的β^{654} ESCs 和野生型的 ESCs。纯合子β^{654} ESCs 的获得弥补了没有纯合子小鼠模型研究的缺陷，对纯合子β^{654} ESCs 进行基因编辑等方法的治疗性研究将更加有效客观地评估治疗方案的有效性。

【关键字】地中海贫血；胚胎干细胞；纯合子；杂合子；珠蛋白

人脑类器官来源间充质干细胞促进阿尔兹海默症小鼠功能恢复的模型研究

谢芳莘、黄恩霞、唐诗丽、薛原、张勇刚

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【摘要】目的 探索人多能干细胞(Human pluripotentstem cells,hPSCs)在体外三维培养条件下产生的间充质干细胞(Mesenchymal stem cells,MSCs)是否可改善阿尔兹海默症(AD)模型小鼠。方法 1)N-MSCs 的获得：从 hPSCs 向前脑类器官诱导分化过程中分离出神经源性间充质干细胞（N-MSCs)，并通过形态学检测、流式检测和三向分化检测进行鉴定。2)与其他 MSCs 比较：对 N-MSCs 和其他来源的 MSCs（如牙间充质干细胞、脐带间充质干细胞、骨髓间充质干细胞等)进行比较，分析 N-MSCs 独有特征。3)N-MSCs 对 AD 小鼠行为学影响：将 28 周龄 AD 小鼠随机分组进行细胞注射治疗，每次尾静脉注射 0.2 mL 细胞悬液（1x106 个/mL)，连续 8 周。治疗后进行行为学检测。治疗完成后进行行为学检测(n-9)。4)组织学证据：通过免疫荧光染色，观察 AD 小鼠海马齿状回区域的新生神经元、神经元末端树突棘、神经干/祖细胞数量、淀粉斑块和小胶质细胞，评价细胞水平改善程度。5)组学数据分析：初步探索 N-MSCs 改善 AD 的机制。结果 1)N-MSCs 鉴定：N-MSCs 与脐带 MSCs 高度相似，符合 MSCs 鉴定标准。2)差异基因分析：N-MSCs 与其他 MSCs 相比，差异基因多与神经营养、炎症反应等高度相关。3 行为学影响：与 PBS 对照组相比，脐带 MSCs 治疗组和 N-MSCs 治疗组小鼠的学习和记忆能力均改善。旷场实验中，N-MSCs 治疗组小鼠更加大胆活泼；新物体识别实验中，脐带 MSCs 组和 N-MSCs 组探索新物体得分更高；条件恐惧实验中，N-MSCs 组小鼠表现出更强的恐惧学习能力；巴恩斯迷宫实验中，N-MSCs 组小鼠显示更强的学习能力和长时记忆效果。4)组织学分析：AD 小鼠脑海马区切片显示，N-MSCs 组新生神经元长度最长、复杂程度更高；齿状回区域神经干细胞最多；神经元末端成熟蘑菇型树突棘数量最多、树突密度最大，差异均具有统计学意义。N-MSCs 治疗组脑海马 DG 和 CAI 区域大面积（>100 μm2) 淀粉斑块数量最少（P<0.05)，小胶质细胞形态趋于非激活态的数量更多，复杂程度更低、分枝长度更短、分枝数量更少。5)机制探索：N-MSCs 可能通过调节炎症状态和增强神经营养作用来改善 AD 症状。结论 与脐带 MSCs 相比，N-MSCs 能更好地改善神经退行性疾病 AD 症状。

【关键字】人多能干细胞；脑类器官；间充质干细胞；AD

tRNAphe 的 Wybutosine 低修饰激活 HERVK 并损害神经元分化

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【摘要】脑性瘫痪是最常见的婴幼儿运动障碍性疾病，是指一组因中枢神经系统早期发育异常导致的持续存在的运动障碍和姿势异常症候群，常伴有全面发育迟缓/智力障碍、癫痫、语言障碍、视觉障碍和自闭症谱系障碍等。目前已知的关于脑瘫病因的遗传和分子信息不足，阻碍了精准医疗时代脑瘫患者的预防、预后和治疗的改善。在我们前期工作中，鉴定了新的脑瘫相关基因 TYW1，其正常情况下其编码的蛋白在 $tRNA^{phe}$ 的 37 位产生超修饰鸟苷，可稳定密码子-反密码子的结合，但其缺失会促进核糖体移码，从而降低翻译的准确性。进一步在斑马鱼和小鼠模型中证实了 TYW1 缺陷会阻碍神经元的增殖和迁移，导致小头畸形、运动和认知功能障碍。因此，蛋白质的正确翻译对神经元增殖至关重要。但 TYW1 功能缺失如何影响神经细胞命运尚未明确。我们的研究首先证明了 TYW1 的缺失阻断了 $tRNA^{phe}$ 中 OHyW 的形成，从而影响了 UUU 密码子的翻译效率。当 TYW1 敲除时，小鼠神经元和类脑器官都表现为神经分化异常。我们发现，在 TYW1 敲除的人胚胎干细胞中 HERVK 异常激活。通过检测介导内源性逆转录病毒组蛋白甲基化的蛋白我们发现，富含 UUU 密码子的 SMARCAD1 表达量显著下调，而 SMARCAD1 回补可以纠正 HERVK 异常激活和神经元分化异常的表型。综上所述，TYW1 缺失通过下调 SMARCAD1 导致 hESC 中 HERVK 异常激活，从而损害神经元分化。

【关键字】TYW1：$tRNA^{phe}$：HERVK：脑类器官;神经增殖和分化

核仁纤维蛋白凝集物通过 rRNA 加工的早期控制驱动急性髓系白血病的发展

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【摘要】急性髓系白血病（AML）难治、易复发的特点仍是患者无病生存的重大障碍，针对白血病干祖细胞（LSPC）开发新的有效药物靶点仍是亟需解决的关键问题。RNA 结合蛋白（RBPs）在 AML 中起着至关重要的作用。虽然在 AML 中已经识别出特定的相分离能力的 RBPs，但它们的凝集物形成对 AML 白血病发生的影响以及相分离靶向药物的治疗潜力尚未被充分探讨。在我们的高通量活体 CRISPR RBP 筛选中，核仁纤维蛋白（FBL，Fibrillarin）被确定为一个关键的 AML 生长相关核仁蛋白，它在 AML 病人样本中异常高表达，与 AML 的发生发展密切相关，然而对正常脐带造血干细胞影响较小。通过功能结构域筛选发现 FBL 主要通过其相分离结构域而不是甲基转移酶或乙酰化结构域来调节 AML 细胞生长、分化、存活以及 LSPC 的自我更新。进一步地利用超高分辨率显微成像、RNA-FISH、RiboMeth-Seq、Northern blot、翻译组测序（polysome profiling-seq）等实验发现，这些相分离结构域具有特定的“序列语法”特征，协调驱动核仁的形成和前体 rRNA 的早期加工（包括移动扩散、切割和甲基化修饰），最终增强了包括 JAK-STAT 信号通路中的关键致癌 mRNA 的翻译。另外，我们发现 CGX-此外，研究还发现，药物 CGX-635，一种已知的 AML 治疗药物，能够直接作用于 FBL，改变其相分离行为，有效抑制 AML 细胞的生长和 LSPC 的自我更新能力。这表明 CGX-635 可能通过靶向核仁蛋白而非组装的核糖体来实现一种新的抗白血病机制。该项研究不仅增进了我们对核仁蛋白在 AML 发展中的作用的理解，还强调了开发针对 FBL 的相分离调节剂作为 AML 治疗方法的潜力。

【关键字】急性髓系白血病；相分离；核仁纤维蛋白；核仁；翻译

The role of immune cell death in spermatogenesis and male fertility

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【摘要】The male reproductive system provides a distinctive shield to the immune system, safeguarding germ cells from autoimmune harm. The testis in mammals creates a unique immunological setting due to its exceptional immune privilege and potent local innate immunity. which can result from a number of different circumstances, including disorders of the pituitary gland, germ cell (GC) aplasia, and immunological elements. Apoptosis, or programmed cell death (PCD), is essential for mammalian spermatogenesis to maintain and ensure an appropriate number of germ cells that that correspond with the supporting capability of the Sertoli cells. Apoptosis is substantial in controlling the number of GCs in testis throughout spermatogenesis, and any dysregulation of this process has been linked to male infertility. There is number of evidence about the potential of PCD in designing novel therapeutic approaches in the treatment of infertility. A detailed understanding of PCD and the processes that underlies immunological infertility can contribute to the progress in designing strategies to prevent and treat male infertility. This review will provide a summary of the role of immune cell death in male reproduction and infertility, and describe the therapeutic strategies and agents for treatment based on immune cell death.

【关键字】Apoptosis, Spermatogenesis, Infertility, Immune privilege, Necroptosis, Ferroptosis

鞘内递送 circPTPRN2 可改善 ALSG93A 小鼠的运动功能

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【摘要】ALS 是以运动神经元退变为特征的致命性神经退行性疾病，复杂的致病机制极大限制了 ALS 治疗策略的开发。目前全球获 FDA 批准用于 ALS 治疗的药物只有四种，分别是化学药物利鲁唑、依达拉奉、Relyvrio 和基因治疗药物 Tofersen，但各项药物仍未出现振奋人心的临床疗效。因此，开发有效的 ALS 治疗策略成为了目前亟待解决的问题。我们利用 ALS SOD1 突变来源的 iPSC 分化至运动神经元建立 ALS 疾病模型，利用 TALEN 基因编辑技术纠正突变基因建立了基因背景完全一样的正常对照系，通过将两种运动神经元进行全转录组测序发现了潜在治疗靶点环状 RNA—CircPTPRN2。我们的研究发现在携带 ALS 突变基因 SOD1 的运动神经元中上调该 CircRNA 能够有效缓解线粒体功能障碍与代谢异常。此外，我们体内研究发现利用腺相关病毒在 ALS G93A 小鼠模型中过表达 CircPTPRN2，能够延缓 ALS 小鼠运动功能障碍，并展现出延长 ALS 小鼠生存期的趋势。因此，CircPTPRN2 有望成为未来治疗 ALS 的有效临床治疗靶点。

【关键字】肌萎缩侧索硬化症，环状 RNA，线粒体功能障碍，基因治疗

中心体蛋白 AKNA 促进成年小鼠的新生神经元成熟和记忆灵活性

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【摘要】Aim: Neurological disorders and aging are closely related to adult neural stem cells (NSC) lineage progression and adult-born neurons-mediated memory flexibility in the hippocampus. However, the underlying mechanism needs to be further clarified. AKNA is involved in a variety of disorders such as cancers, inflammations and immune disorders. It is reported that AKNA regulates the delamination and retention of NSC in the embryonic subventricular zone (SVZ). However, how AKNA governs adult NSC lineage progression to further conduct hippocampal-related memories remains unknown.

Methods: Using CRISPR-Cas9 gene knockout technique, combining with NSC primary culture, viral delivery system, RNAseq, CoIP-MS analysis, and animal behavioral tests, we investigated how AKNA regulates adult hippocampal NSC lineage progression and hippocampal-related memory flexibility.

Results: Knockout of AKNA remarkably inhibited the proliferation and differentiation of primary cultured NSC in vitro. Knockout of AKNA especially in mouse hippocampus significantly retarded adult hippocampal NSC lineage progression into maturation of adult-born neurons in Rosa26-LSL-Cas9-EGFP mice. These results reveal that AKNA is vital to adult hippocampal NSC lineage progression. Furthermore, we discovered that knockout of AKNA induced hippocampal-related depressive-like behaviors and impaired flexibilities of spatial and fear memory in Rosa26-LSL-Cas9-EGFP mice. Surprisingly, centrosome-anchored AKNA but not cytoplasmic-anchored AKNA rescued AKNA deficiency-induced those.

Conclusion: This study newly discovers that centrosome-anchored AKNA but not cytoplasmic-anchored AKNA triggers hippocampal NSC lineage progression into mature adult-born neurons and facilitates spatial and fear memory flexibilities in adult mice. The study provides new proofs for functions of centrosome protein in neurological disorder-related learning and memory and new potential therapeutical targets for CNS diseases.

【关键字】AKNA, Adult hippocampal NSC lineage progression, Adult-born neurons, Memory flexibility

ADSCs 的细胞外囊泡通过 Gbp3 调控抑制缺血性卒中诱导的焦亡：作用于 NLRP3/GSDMD 信号通路

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【摘要】Background and Objective: Mounting data indicates that extracellular vesicles (EVs) have the potential to improve the injury after a stroke. Pyroptosis is recognized as a novel cell death form between necrosis and apoptosis, the main pathological manifestations of which are nuclear consolidation, cell membrane pore formation and rupture, cell swelling, and inflammatory reactions caused by the release of cell contents into the extracellular space. Studies have shown that pyroptosis is widespread in the course of ischemic stroke, and that it is closely related to the development of neuroinflammation and brain tissue damage following cerebral ischaemia. We aimed to ascertain the therapeutic implications and possible molecular processes of EVs obtained from adipose-derived stem cells (ADSCs) in inhibiting cellular pyroptosis in ischemic stroke.
Materials and methods: The investigation employed transient middle cerebral artery occlusion (tMCAO) rat model and a BV2 of oxygen-glucose deprivation/reoxygenation (OGD/R) to ascertain ADSCs-EVs implications on inflammation and pyroptosis as assessed by neurological deficit scores, TTC staining, IHC, HE, CCK8, WB, ELISA, and immunofluorescence. RNA-Seq was performed on BV2 cells in the control, OGD/R, and OGD/R + ADSCs-EVs groups. Using sequencing data analysis, in the OGD/R group, we screened the upregulated genes regulated by EVs, overlapped with 74 pyroptosis-related genes, and identified Guanylate-binding protein 2(Gbp2) and Guanylate-binding protein 3 (Gbp3) as key genes. Following the validation of the sequencing results in vivo and in vitro, Gbp3 was selected for further study. To test its regulatory effects on inflammation and pyroptosis, Gbp3 was knocked down and overexpressed in vitro.
Results: The administration of ADSCs-EVs resulted in a significant enhancement in neurological involvment scores and a reduction in infarct volume in rats with tMCAO. They were also protective against BV-2 cells after OGD/R. In vivo and in vitro, ADSCs-EVs inhibited inflammatory response and pyroptosis after stroke. The outcomes of the RNA-Seq data analysis manifested that the protective implications of EVs after stroke are mediated by the modulation of inflammation-related mechanisms. Moreover, treatment with EVs led to a significant reduction in Gbp3 expression in post-ischemic brain tissue and cells. When Gbp3 was knocked down, the expression of inflammatory molecules and proteins linked to pyroptosis had a significant decline. When Gbp3 was overexpressed, the opposite results were obtained.
Conclusion: We succeeded in extracting ADSCs-EVs, verified the therapeutic consequences of ADSCs-EVs in the rat tMCAO model and the in vitro BV2 fine OGD/R model, and investigated the molecular mechanisms. Our outcomes emerge that ADSCs-EVs could inhibit pyroptosis and inflammation following stroke in vitro and in vivo. Moreover, we found that ADSCs-EVs attenuate post-stroke inflammation and pyroptosis by inhibiting Gbp3 and modulating the NLRP3/GSDMD pathway, opening a new direction for stroke therapy.
【关键字】 Ischemia, Extracellular vesicles, Pyroptosis, Adipose-derived stem cells, Gbp3

卒中后神经发生研究现状

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【摘要】身体的组织损伤在发生损伤后的一段时间往往伴随着区域性的再生修复，这种现象在各个物种的各个器官都有报道。同身体的其他组织一样，卒中后伴随着脑部 SVZ 区和 SGZ 区爆发式的神经再生以及一系列相关的细胞信号通路的激活。有研究表明在卒中后爆发式新生的神经元往往不能够具有正常的突触形态，这可能是受到异常的细胞信号影响的原因，一些学者将这些新生神经元错误的形态发生视作是引起认知障碍发生的原因。事实上，即使卒中后伴随着局部脑区的新生神经元数量激增，受限于神经再生的范围和程度，起对整个脑区的神经环路影响也是十分有限的。卒中的神经再生到底在整个病理过程中发挥了怎样的利弊作用？触发神经再生的机制是什么？这种信号是如何从梗死灶传递到干细胞发生区的？这些再生的细胞的命运又是如何被决定的？调整神经再生的速率能否对病后的恢复产生有益的影响？这些问题都值得我们深入探索。

【关键字】 卒中 神经发生

基于新型无菌免疫人源化小鼠研究肠道菌群对人免疫重建的作用及其机制

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【摘要】肠道菌群在调节免疫稳态和免疫重建中起重要作用，肠道菌群失衡与造血干细胞移植后患者的免疫重建障碍和不良预后相关。由于研究手段的限制，肠道菌群与免疫重建的机制及其因果关系尚不明确。无菌（GF）动物是研究微生物组与健康和疾病因果关系的金标准。本研究中，我们结合无菌小鼠和 BLT 人源化手术，开发了无菌免疫人源化小鼠（GF-BLT）。基于此模型，我们通过菌群定植构建了 SPF 饲养来源的鼠源肠道菌免疫人源化小鼠（MuM-BLT）和健康志愿者来源的人源肠道菌免疫人源化小鼠（HuM-BLT），探索不同来源的肠道菌群对免疫重建过程中的动态影响。从外周血水平深入到重点器官和组织水平内的免疫细胞变化，结果显示，不同肠道微生物背景的人源化小鼠调节多种组织器官内的免疫细胞分布。受到影响的主要器官包括脑、淋巴结和肠道，主要受影响的细胞类型为 T 细胞和髓系细胞。本研究为探讨肠道微生物与干细胞移植后人免疫重建的互作提供了有力的动物模型，也为利用菌群干预的方式促进人免疫系统稳态提供了理论和实验依据。

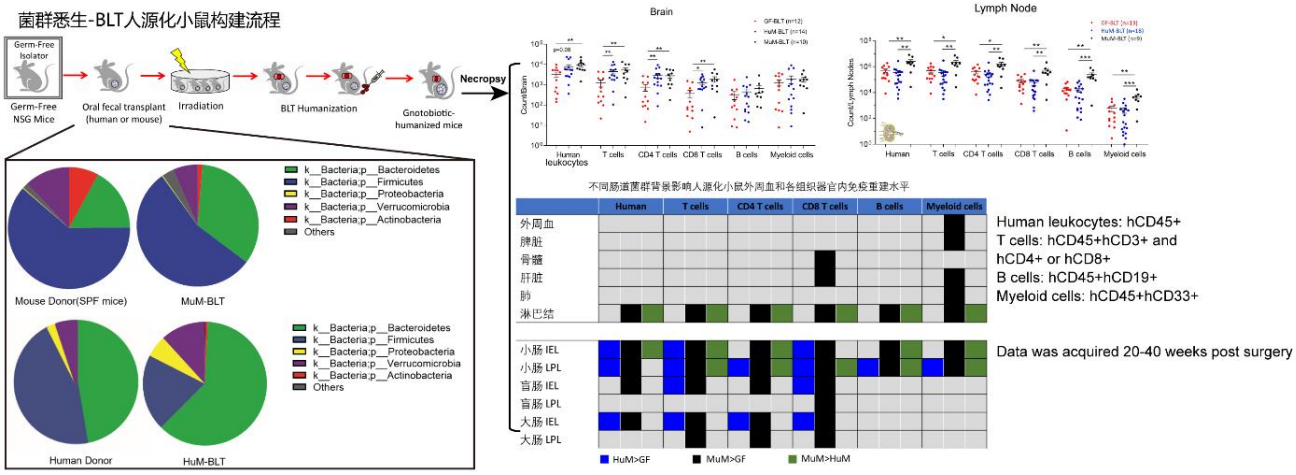

Fig. 1 Gut Microbiota Regulates Immune Cell Reconstitution In The Gnotobiotic-Humanized Mice

【关键字】无菌小鼠；肠道菌群；人源化小鼠；免疫重建

活体显微成像在干细胞治疗研究中的应用

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IVIM Technology

【摘要】活体显微成像技术在干细胞研究中扮演着至关重要的角色，能够提供动态且具有空间分辨率的细胞行为和交互作用资讯。本研究利用活体显微成像技术，探讨内皮祖细胞（endothelial progenitor cells, EPCs）在缺血性后肢模型和肝损伤模型中的治疗潜力及血管重塑能力。

在实验一中，我们评估了 EPCs 在小鼠后肢缺血模型中介导的血管生成作用。动脉结扎后将 EPCs 注射到一侧后肢，并对随后的血管变化进行成像。结果显示，从第 14 天开始观察到显着的血管生成和结构变化，这些发现强调了活体显微成像技术在准确监测 EPCs 分布和功能中的重要性。为进一步证实 EPCs 的细胞行为，我们使用绿色荧光蛋白（GFP）标记的 EPCs，显示了细胞在受损组织中的迁移、分布及血管内皮的整合，从而提供了更详细的细胞动态信息。

在另一项实验中，通过生物打印技术，将小鼠的化学衍生肝细胞（mouse chemically derived hepatic progenitors, mCdH）和内皮细胞（mouse endothelial cells, mECs）组合成三维的球状体结构，将这些球状体然后注射到 FRG-/-基因敲除小鼠的肝脏中，以观察这些打印的球状体在缺失某些基因的小鼠肝脏中的行为和功能，以及它们是否能够补偿或替代缺失基因导致的功能缺陷。活体显微成像结果显示，随着时间的推移，了解植入细胞与宿主血管之间的动态交互作用，通过不同时间点的成像，揭示了球状体内细胞的分化过程以及新生血管网络的形成，进一步强调了内皮细胞在血管重塑中的关键角色。

通过这些实验，本研究强调了活体显微成像作为推进我们对干细胞疗法及其在再生医学中机制理解的不可或缺的工具。这些结果不仅证实了 EPCs 和生物打印球状体在促进血管生成和组织修复中的潜力，还展示了活体显微成像在评估和优化这些疗法中的应用价值。

二、多能性与细胞命运决定

长链非编码 RNA LHX1-DT 通过 H2A.Z 介导的 LHX1 转录激活调控心肌细胞分化

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【摘要】**Background and Purpose** Pluripotent stem cells (PSCs) can be induced to differentiate into cardiomyocytes in vitro to replace the damaged myocardial tissue and have great promise for clinical application. However, the regulatory factors and mechanisms of myocardial differentiation have not been fully elucidated yet, which restricts their development in clinical applications. In recent years, long non-coding RNA (lncRNA) has been reported to play an important regulatory role in cardiovascular diseases and cardiac development. The aim of this work is to investigate the key regulatory role and mechanism of LHX1-DT in the differentiation process from embryonic stem cells(ESCs) to cardiomyocytes, provide new insights into the mechanism of early development of the human heart, and provide new therapeutic targets for cardiogenic diseases.

Methods (1) To establish an in vitro model of differentiation of ESCs into cardiomyocytes. (2) qRT-PCR was used to detect the mRNA expression levels of marker genes and screen candidate lncRNAs. (3) Bioinformatics analysis of LHX1-DT co-expressed genes in mesoderm. (4) The differentiation efficiency of cardiomyocytes was detected by flow cytometry. (5) Analysis of mesodermal gene expression profiles by RNA-seq. (6) LHX1-DT knockout ES cell lines were constructed by CRISPR/Cas9 technology. (7) Immunofluorescence was used to detect the expression levels of pluripotency marker proteins SOX2 and OCT4, cardiac cell marker proteins cTnT and α-actinin. (8) FISH assay was used to detect the intracellular distribution of LHX1-DT and LHX1. (9) Western blot was used to detect the expression of LHX1 and H2A.Z. (10) ChIP assay showed that H2A and H2A.Z were enriched in the LHX1 promoter region. (11) The interaction between LHX1-DT and PHF6 protein was detected by RIP assay and RNA-pull down assay. (12) FISH assay was used to verify the binding of PHF6 to H2A.Z.

Results (1) The expression level of LHX1-DT was significantly increased at the mesoderm stage. RNA sequencing analysis showed that the differentially expressed genes after knockdown of LHX1-DT were related to cell differentiation and development. Knockdown of LHX1-DT inhibited the differentiation of ESCs into mesoderm, cardiac progenitor cells and cardiomyocytes.

(2) LHX1-DT and LHX1 colocalize in the nucleus, knockdown of LHX1-DT can reduce the expression of LHX1, knockdown of LHX1 can inhibit the differentiation of ESCs into mesoderm and cardiomyocytes, overexpression of LHX1 in LHX1-DT knockout cell line can rescue the phenotypic changes caused by knockout of LHX1-DT.

(3) At the mesodermal stage, the histone variant H2A.Z was enriched in the promoter region of LHX1, while the conventional histone H2A was decreased. Knockdown of H2A.Z reduced the expression of LHX1.

(4) Knockdown of LHX1-DT inhibited the enrichment of H2A.Z in the promoter region of LHX1 and promoted the enrichment of H2A, but did not affect the expression of H2A.Z. RNA pulldown and RIP experiments confirmed that LHX1-DT could directly bind to PHF6 at the mesodermal stage, and PHF6 also bound to H2A.Z.

Conclusions LncRNA LHX1-DT is highly expressed in the mesoderm, and binds to PHF6 protein to regulate the conversion of H2A to H2A.Z enriched in the LHX1 promoter region to activate the transcription of LHX1, thereby promoting the differentiation of ESCs into cardiomyocytes.

【关键字】LHX1-DT, LHX1, ESCs, Cardiomyocyte differentiation

Mechanistic investigation of Zfp352 on early embryonic development regulation in vivo and in vitro

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【摘要】Early embryogenesis is orchestrated by transcription factors that regulate the genome activation (ZGA) process to establish totipotency and then specify the lineages forming the embryo. As little is known about how these factors trigger totipotency network, we focus on the gene highly expressed during ZGA. In this study, we use molecular, cellular, and genetic approaches to show, unexpectedly, that Zfp352 alone plays an important role in endogenous retroviruses activation in embryonic stem cells (ESCs). Specifically, overexpression of Zfp352 is sufficient to induce ZGA gene and transposable elements (TEs). Mechanistically, overexpressing of Zfp352 will modify the enrichment of H3K9me3 to degrade pluripotent protein and finally activate MERVL/2C gene expression. Next, we generated Zfp352 knockout (KO) mouse lines. Unexpectedly, we found that Zfp352 zygotic KO embryos can survive to adulthood. However, we uncover an unanticipated of the Zfp352 in the regulation of meiosis during spermatogenesis. Thus, contrary to the key function of Zfp352 in inducing 2C-like cells, our data indicate that Zfp352 is essential for male fertility in mice.

【关键字】Zfp352, 2-Cell like cells, Zygotic genome activation, Spermatogenesis

The conserved role of RNA splicing in regulating pluripotency-to-totipotency transition

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【摘要】RNA splicing is one of the key process in the regulation of gene expression, and its accuracy and efficiency is crucial for maintaining cell homeostasis. Studies have shown that specific RNA splicing events can regulate the activity of key transcription factors and determine the choice of cell fate decision during stem cell pluripotency maintenance and differentiation. Firstly, we collected multi-species preimplantation embryo sequencing data and found several conserved pathways during the zygotic genome activation (ZGA) stage. Secondly, in the embryo and cell experiments, ZGA retention caused by splicing repression is verified by activating DNA damage pathway. This mechanism is conserved in multiple species. Therefore, our results indicate that RNA splicing repression can promote DNA damage and further activate totipotent genes expression, thereby affecting cell fate in the cells and early embryo, and this mechanism is conserved in multiple species.

【关键字】RNA splicing, DNA damage, Totipotent genes, conserved

Single-cell 3D genome structure reveals distinct human pluripotent states

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【摘要】Background: Pluripotent states of embryonic stem cells (ESCs) with distinct transcriptional profiles affect ESC differentiative capacity and therapeutic potential. Although single-cell RNA-sequencing has revealed additional subpopulations and specific features of naïve and primed human pluripotent stem cells (hPSCs), the underlying mechanisms that regulate their specific transcription and that control their pluripotent states have remained elusive.

Results: By single-cell analysis of high-resolution, three-dimensional (3D) genomic structure, we herein demonstrated that remodeling of genomic structure was highly associated with the pluripotent states of human ESCs (hESCs). The naïve pluripotent state was featured with specialized 3D genomic structures and clear chromatin compartmentalization that was distinct from the primed state. The naïve pluripotent state was achieved by remodeling the active euchromatin compartment and reducing chromatin interactions at the nuclear center. This unique genomic organization was linked to enhanced chromatin accessibility on enhancers and elevated expression levels of naïve pluripotent genes localized to this region. In contradistinction, the primed state exhibited intermingled genomic organization. Moreover, active euchromatin and primed pluripotent genes were distributed at the nuclear periphery, while repressive heterochromatin was densely concentrated at the nuclear center, reducing chromatin accessibility and the transcription of naïve genes.

Conclusions: Our data provide insights into the chromatin structure of ESCs in their naïve and primed states, and we identified specific patterns of modifications in transcription and chromatin structure that might explain the genes that are differentially expressed between naïve and primed hESCs. Thus, the inversion or relocation of heterochromatin to euchromatin via compartmentalization is related to the regulation of chromatin accessibility, thereby defining pluripotent states and cellular identity.

【关键字】human embryonic stem cells, pluripotency, naive, primed, genome structure, chromatin accessibility

Lamin B1 调控红系异染色质构象动态变化及分化过程

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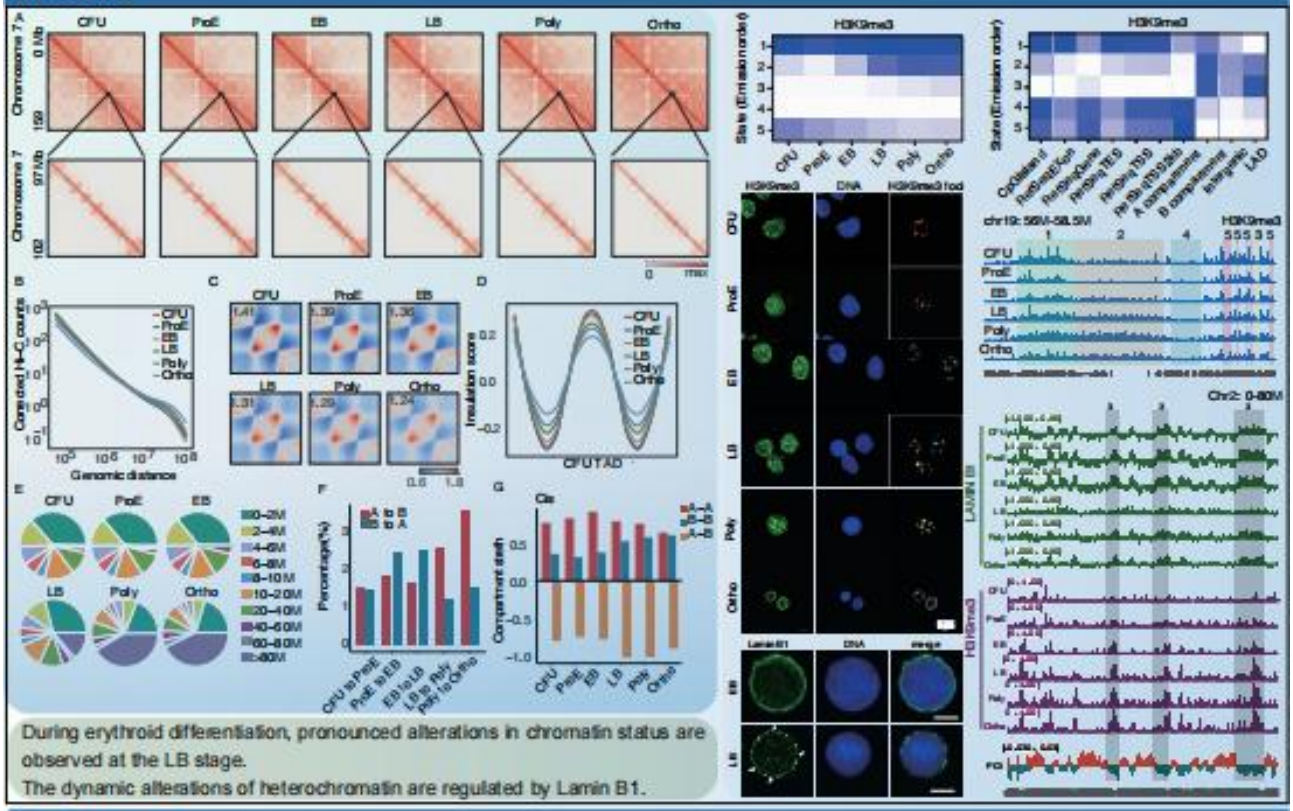
ABSTRACT

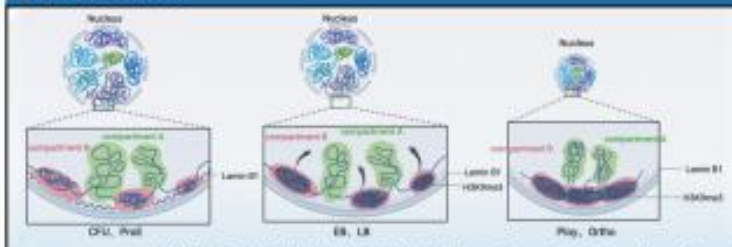
Chromatin condensation is a crucial step in the maturation process of red blood cells. we explored the distribution changes of chromatin modifications during erythroid differentiation, the dynamic regulatory factors of Lamin B1, and their relationship with changes in chromatin structure. In combination with a mouse model, we revealed the important role of the nuclear lamina protein Lamin B1 in erythroid differentiation and the molecular mechanisms regulating chromatin condensation.

RESULTS



During erythroid differentiation, pronounced alterations in chromatin status are observed at the LB stage.
The dynamic alterations of heterochromatin are regulated by Lamin B1.

CONCLUSION



During the early stages of erythroid differentiation, Lamin B1 ensures a normal differentiation process. In the late basophilic stage, the absence of Lamin B1 leads to the disruption of Lamina-Associated Domains (LADs), which in turn promotes changes in higher-order chromatin structure mediated by heterochromatin.
That is, at different stages of erythroid differentiation, the nuclear lamina protein Lamin B1 exerts precise regulatory control and is an important regulatory factor for higher-order chromatin structure.

H3K9me3 established, lamin B1 deleted chromatin condensated, nuclear volume decreased

血清铁通靶向 ACLS4 诱导 CAR-T 细胞终末分化的作用及机制研究

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【摘要】CAR-T（Chimeric antigen receptor-T）细胞疗法已被认为是最具临床转化价值的细胞免疫疗法，目前全球获 FDA 认证的 CAR-T 产品已达 7 款，均应用于血液恶性肿瘤的治疗。尽管疗效显著，但治疗后的疾病复发以及 CAR-T 体内持久性差等问题仍亟需解决。本课题组早期研究表明 CAR-T 细胞在病人体内的持久性与其干性维持相关。而体内微环境对 CAR-T 细胞干性维持作用研究较少，尤其是离子环境的改变。

本项目对 88 例恶性血液肿瘤病人进行了血清非靶代谢及离子代谢测序分析，结果显示 CAR-T 细胞在死亡时所处血液环境发生了脂质代谢及铁离子代谢异常；结合 4 例病人的单细胞测序结果，我们发现铁离子相关基因及信号通路显著富集。进一步对病人流式检测验证表明铁死亡 Marker：LipidROS 及细胞内游离二价铁离子均在细胞死亡时显著升高，提示 CAR-T 细胞受到血清铁的胁迫并发生铁死亡现象。随后，我们构建了稳定的高铁小鼠模型，并注入 CAR-T 细胞及肿瘤细胞，数据结果显示高浓度的血清铁显著的抑制了 CAR-T 对肿瘤的杀伤作用。体外 CAR-T 培养中添加铁离子后相关 CAR-T 功能检测发现 20μM（正常人类血清铁离子浓度约为 10μM-30μM）铁离子即可显著性促进 CAR-T 的终末分化，诱导细胞耗竭；此外，通过靶向花生四烯酸代谢途径的 ACSL4 环氧酶，显著的抑制了细胞终末分化，并在小鼠体内模型提高了治疗效果，延长了小鼠生存时间。本研究从临床问题出发，揭示了 CAR-T 体内治疗中的铁离子诱导的终末分化细胞毒性作用，并生产了 CAR-T-ACLS4-KO 的新型产品，并在小鼠体内提升了 CAR-T 疗效。本研究奠定了 CAR-T 铁离子领域的研究基础，具备良好的临床转化价值。

【关键字】嵌合抗原受体 T 细胞、血清铁、花生四烯酸代谢途径

Zscan4 介导共抑制因子复合物的泛素化降解，以促进 2C 样细胞的染色质可及性

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【摘要】Zygotic genome activation (ZGA) occurs in two-cell (2C) embryos, and a 2C-like state also is activated in sporadic (~1%) naïve embryonic stem cells (ESCs) in mice. Elevated chromatin accessibility is critical for the 2C-like state to occur, yet the underlying molecular mechanisms remain elusive. Zscan4 exhibits burst expression in 2C embryos and 2C-like cells (2CLCs). Here we show that Zscan4 mediates chromatin remodeling to promote the chromatin accessibility for achieving the 2C-like state. Through co-immunoprecipitation/mass spectrometry (Co-IP/MS), we identified that Zscan4 interacts with the co-repressors Kap1/Trim28, Lsd1, and Hdac1, also with H3K9me3 modifiers Suv39h1/2, to transiently form a repressive chromatin complex. Then, Zscan4 mediates the degradation of these chromatin repressors by recruiting Trim25 as an E3 ligase, enabling the ubiquitination of Lsd1, Hdac1, and Suv39h1/2. Degradation of the chromatin repressors promotes the chromatin accessibility for activation of 2C-like state. These findings reveal the molecular insights into the roles of Zscan4 in promoting full activation of 2C-like state.

【关键字】Zscan4, Chromatin accessibility, Zygotic genome activation, 2C-like state, Ubiquitination.

核糖体生物合成调控生血内皮细胞向造血干细胞转化

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【摘要】胚胎主动脉内皮细胞向造血转化的过程中，一部分胚胎主动脉内皮细胞首先特化为生血内皮细胞，并最终分化为造血干细胞。早前研究发现，与邻近的动脉内皮细胞相比，具有造血干细胞命运的生血内皮中显著富集了核糖体生物合成过程。然而，核糖体生物合成在生血内皮中的功能仍不清晰。本研究中，我们发现核糖体生物合成活性在生血内皮细胞往造血干细胞转化过程中显著增强。抑制核糖体生物合成完全阻断了外植体培养中造血干细胞的产生。此外，破坏核糖体生物合成选择性地中断了生血内皮细胞而不是造血干细胞前体往造血干细胞分化的潜能。机制上，抑制核糖体生物合成显著抑制了生血内皮细胞而非造血干细胞前体的细胞周期进程。进一步的研究表明，在生血内皮细胞特化时，造血转录因子 Runx1 显著地结合到核糖体生物合成相关基因的位点上，从而促进了这一生物学过程。综上所述，我们的研究表明核糖体生物合成在胚胎造血干细胞生成中发挥了不可或缺的作用，研究也为体外再生造血干细胞提供了新的线索。

DUX 诱导 mESCs 向胚外内胚层分化的机制探索性研究

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【摘要】目的：探索双同源盒 (double homeobox, DUX) 蛋白对小鼠胚胎干细胞 (mouse embryonic stem cells, mESCs）向胚外内胚层（extraembryonic endoderm，XEN）分化潜能的影响及可能的作用机制。

方法：使用慢病毒体系在 mESCs 中构建过表达 DUX 细胞系，检测其在多能状态和自然分化状态下各胚层标志性基因的表达；挖掘公共 RNA-seq 数据，进一步明确 DUX 对 mESCs 向胚外内胚层分化的影响；通过对差异基因的功能及通路富集分析（Gene Set Enrichment Analysis, GSEA），找出 DUX 作用的信号通路；深入分析已有的 ChIP-seq 数据，探究 DUX 的潜在靶基因。

结果：分子生物学实验显示过表达 DUX 后可有效维持 mESCs 的多能性，与公共 RNA-seq 数据分析结果一致；继而对差异基因分析，发现内胚层基因出现特异性上调；诱导 mESCs 自然分化后，RT-qPCR 检测实验表明 XEN 标志基因（Gata4、Gata6、Sox17）的 mRNA 表达出现显著上调，而中外胚层基因没有特异性变化。GSEA 富集分析结果指出 DUX 可能激活了视黄醇代谢信号通路，ChIP-seq 数据解析进一步揭示 DUX 与视黄酸受体占据相似的位点，可激活下游与胚外内胚层发育相关的靶基因。

结论：DUX 与视黄酸信号通路有着密切关联，预示其激活了视黄酸信号通路，从而促进 mESCs 倾向胚外内胚层分化。

【关键字】胚胎干细胞；胚外内胚层细胞；DUX；视黄酸信号通路。

Application of human umbilical cord derived mesenchymal stem cell-derived extracellular vesicles for in vitro expansion of umbilical cord hematopoietic stem cells

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【摘要】Hematopoietic stem cells (HSCs) are adult stem cells in the blood system. The application of mesenchymal stem cells (MSCs) for in vitro expansion of hematopoietic stem cells (HSCs) can effectively maintain the original characteristics of HSCs and their long-term reconstruction ability during transplantation. The therapeutic effect of MSCs mainly relies on paracrine mechanisms, including exosome secretion. The purpose of this study is to investigate the effect of umbilical cord derived mesenchymal stem cell exosomes (MSCs exosomes) on the in vitro expansion of umbilical cord hematopoietic stem cells.CD34+cells were selected from human umbilical cord blood and cultured in vitro for 10 days. The expanded HSCs were analyzed by flow cytometry and subjected to primitive colony forming unit (CFU) assay. Primary mesenchymal stem cells were obtained from human umbilical cord using tissue direct adhesion method and passaged to P4-P5 for cell identification. When the cell fusion degree reached 60%~70%, serum-free medium was used to continue culturing for 72 hours, and the culture supernatant was collected. The obtained culture supernatant was centrifuged at ultra-high speed to obtain extracellular vesicles of mesenchymal stem cells.Compared with the control group, the experimental group supplemented with mesenchymal stem cell extracellular vesicles showed significant increases in total nucleated cells (TNCs), CD34+cells, CD34+CD38-cells, and CD34+CD38-Lin-cell subsets after 10 days of in vitro expansion and culture. In addition, the experimental results of colony forming units showed that the total number of CFUs in the expanded cells of the experimental group was significantly higher than that of the control group.Umbilical cord mesenchymal stem cell exosomes can effectively promote the in vitro expansion of umbilical cord blood hematopoietic stem cells while maintaining their original characteristics.

【关键字】Umbilical cord; Umbilical cord blood; Mesenchymal stem cell; Hematopoietic stem cells; Extracellular vesicles; in vitro amplification

ZSCAN10 在 hESC 向中内胚层分化过程中的作用及机制

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【摘要】人胚胎干细胞（human embryonic stem cells，hESCs）具有无限自我更新（self-renew）和发育的多能性（pluripotency），因而成为研究人胚胎早期发育的良好细胞模型。但在诱导 hESC 向不同胚层谱系或组织细胞类型分化过程中，我们仍难以高效均一地获得目标细胞类型。造成该现状的主要原因之一，则是对调控 hESC 命运决定的关键因子及其作用机制的研究还不够充分。转录因子在细胞命运的决定中发挥关键的作用。ZSCAN10 是具有蛋白质结合结构域（SCAN 结构域）及 DNA 结合序列（Zinc Finger 序列）的转录因子。其在 hESC 中的表达特征和功能还未有报导。在本课题中，我们利用靶向 ZSCAN10 的 siRNA 和 dTAG 诱导降解 ZSCAN10 的系统，发现 ZSCAN10 对 hESC 向中内胚层谱系的正常分化具有调控作用。ZSCAN10 的缺失或过表达分别导致 hESC 向中内胚层谱系分化中关键基因表达的异常升高或降低。结合 RNA-seq、CUT & Tag 和 Co-IP/MS 多组学的机制研究显示 ZSCAN10 在三个层面上参与调控了中内胚层的分化。首先作为转录因子，ZSCAN10 能直接结合中内胚层分化的关键基因 GATA4、GATA6 和 EOMES 的启动子区域。其次，ZSCAN10 能够与 CBX3-TRIM28 结合，共同对中内胚层基因的表达起到抑制作用。此外，在信号通路方面，ZSCAN10 还能够抑制 WNT 信号通路的活性，实现其对中内胚层分化的调控作用。因此，我们发现了一个在 hESC 定向分化为中内胚层谱系过程中起负调控作用的转录因子，揭示了 ZSCAN10 通过直接结合靶基因并招募表观遗传调控因子共同抑制中内胚层分化基因表达的机制，建立了转录因子与重要信号通路之间的联系。这些发现进一步完善了 hESC 命运决定调控网络，并为优化 hESC 体外扩增和定向分化培养条件奠定了基础。

【关键字】人胚胎干细胞，ZSCAN10，中内胚层，CBX3，TRIM28，WNT 信号通路。

单细胞转录组测序解析缺血性脑卒中后小胶质细胞干性改变

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【摘要】一、背景与目的

缺血性脑卒中是一种常见的中枢神经系统疾病，导致脑组织缺氧、坏死，并伴随严重的神经炎症反应。小胶质细胞作为脑内的常驻免疫细胞，在缺血性脑卒中的发生和发展过程中起着至关重要的作用。为了深入了解缺血性脑卒中后小胶质细胞的功能和状态变化，本研究采用单细胞转录组测序技术，系统解析缺血性脑卒中后小胶质细胞的干性改变及其潜在机制。

二、方法

通过建立缺血性脑卒中小鼠模型，提取梗死区域和周边区域的脑组织。利用单细胞分离技术，获取单个小胶质细胞，并进行单细胞 RNA 测序。随后，使用生物信息学分析工具对单细胞转录组数据进行聚类分析、差异表达分析和通路富集分析，以揭示缺血性脑卒中后小胶质细胞的异质性及其干性改变。

三、结果

单细胞转录组测序数据展示了缺血性脑卒中后小胶质细胞的显著异质性。相比于对照组，缺血性脑卒中后的小胶质细胞表现出明显的干性改变，包括增殖能力的增强、表面标志物表达的变化以及与炎症相关基因的上调。

四、结论

本研究通过单细胞转录组测序技术，系统解析了缺血性脑卒中后小胶质细胞的干性改变。研究结果表明，缺血性脑卒中诱导了小胶质细胞的显著异质性和干性改变，这些变化可能通过多种信号通路调控，提示小胶质细胞在脑卒中后的再生和修复过程中具有潜在的靶点价值。未来的研究将进一步验证这些发现，并探索其在临床干预中的应用潜力。

Pseudogenes contribute to the evolution of topological domains across species

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【摘要】The human genome harbors 15,000 pseudogenes, except very few can transcribe non-coding RNAs or encode truncated proteins, a large number of which without transcriptional capacity are functional unknown. Here, we found that pseudogene DNA sequences can form chromatin contacts that act as anchors of chromatin loops and boundaries of topologically associating domains (TADs), many of which proved to be structurally important and essential for survival in human embryonic stem cells (hESCs). Incorporating genetic data, we defined a Hominoidea-specific pseudogene, TUBBP2, which acted as a TAD boundary to maintain the three-dimensional (3D) genome structure and self-renewal capacity in hESCs. TUBBP2 first evolved 18.8 million years ago through retroposition and inherited the CTCF-binding motif from its parent gene TUBB, thus enhancing the strength of TADs at the insertion site in the great ape genome. More amazing, by inheritance from parent genes or sequence variation, certain pseudogenes can introduce additional CTCF binding sequence at their insertion sites to generate species-specific topological domains, which may contribute to species evolution. Overall, we demonstrate the essentiality of pseudogenes in the formation and maintenance of 3D chromatin structure and provide insights into their functions in driving species evolution.

【关键字】Pseudogene, three-dimensional chromatin structure, evolution of 3D genome

OIP5-AS1 调控的 RNA 结合蛋白 HuR 可能参与了人类母源-合子转化过程中母体转录本的降解

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【摘要】Introduction: The maternal-to-zygotic transition is an essential step in the early development of humans, in which maternal gene transcripts undergo extensive degradation and zygotic genes are activated. Dysregulation of maternal transcript degradation may be associated with various reproductive disorders. However, to the best of our knowledge, the exact mechanism of maternal transcript degradation during maternal-to-zygotic transition is still unclear.

Materials and Methods: The oocyte-specific module was identified by weighted gene co-expression network analysis, and enrichment analysis of the genes in this module was performed. Then, the mRNAs bound to the HuR protein were identified in induced pluripotent stem cells by RNA immunoprecipitation sequencing.

Results: Using weighted gene co-expression network analysis, an oocyte-specific module was identified, which was associated with transcription factor binding, protein modification and the cell cycle. Within this module, a maternal long non-coding RNA, OIP5 antisense RNA 1 (OIP5-AS1), was identified. OIP5-AS1 may bind to the RNA binding protein human antigen R (HuR), restricting its availability to other mRNAs, and potentially contributing to maternal transcript degradation during MZT. The physical interaction between OIP5-AS1 and HuR was also validated. RNA immunoprecipitation sequencing identified mRNAs that bind to the HuR protein, and their functions were revealed to be related to transcriptional regulation and the cell cycle.

Conclusions: The HuR protein, regulated by OIP5-AS1, may be involved in maternal transcript degradation and other critical biological processes during maternal-to-zygotic transition in early human embryonic development.

【关键字】Early embryonic development; Long non-coding RNAs; Maternal transcript degradation; RNA binding protein; Maternal-to-zygotic transition

基于单细胞测序分析 CD248+成纤维细胞亚群促进胃癌侵袭转移的作用研究

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【摘要】胃癌（Gastric cancer, GC）是发病率和死亡率均较高的消化道恶性肿瘤，而且近年来其发病率还有呈逐年上升的趋势。早期 GC 或局部 GC 主要通过外科手术予以切除，然而，由于 GC 具有高度侵袭性的特点，在早期即可发生向周围组织的侵袭或扩散至远处脏器，因而 GC 患者的预后较差。因此，深入研究 GC 侵袭和转移的机制对于探寻 GC 更有效的治疗方法具有重要价值。

已有证据表明肿瘤的侵袭和转移不仅仅取决于肿瘤细胞本身的特性，也与其所处肿瘤微环境（Tumor microenvironment, TME）密不可分。TME 中的重要组成成分之一是成纤维细胞，被称之为肿瘤相关成纤维细胞（Cancer-associated fibroblasts, CAFs）。CAFs 受到肿瘤细胞来源的细胞因子的诱导而激活，活化后的成纤维细胞或产生大量胶原蛋白、透明质酸等基质蛋白重塑细胞外基质（Extra Cellular Matrix, ECM）而形成有利于肿瘤细胞侵袭和转移的微环境，或分泌细胞因子、趋化蛋白等激活肿瘤细胞内相应通路进而促进肿瘤细胞自身的侵袭和转移。因此，研究 CAFs 在胃癌侵袭转移中的作用及机制必将为最终揭示胃癌侵袭转移的机制作出重要贡献。

CAFs 具有高度异质性。单细胞转录组学技术的兴起和应用，揭示了不同的 CAF 亚群在不同肿瘤中发挥不同的功能，如促进肿瘤细胞增殖、耐药和代谢等，但究竟是哪群成纤维细胞在促进 GC 侵袭和转移发挥作用却鲜有报道，因此我们基于单细胞转录组数据初步分析了人胃癌 CAF 亚群的全貌，鉴定出一群促 GC 侵袭和转移的 CAF 亚群并验证了其功能。

本课题研究中，我们分析了 GEO 数据库中 29 例原发 GC 组织、11 例正常胃组织、4 例胃癌腹膜转移灶组织和 1 例 GC 患者正常腹膜组织的单细胞转录组测序（single-cell RNA sequencing, scRNA-seq）数据，使用单细胞降维聚类等方法绘制了 GC 及其转移癌的单细胞转录图谱，采用基因富集等方法分析了各主要细胞的功能，鉴定了与 GC 侵袭转移密切相关的成纤维细胞亚群以及它们与临床病理特征之间的关系，并利用细胞培养、Transwell 迁移和侵袭实验等体外实验验证了上述发现。主要结果、结论如下：

绘制了原发胃癌、正常胃组织、胃癌腹膜转移灶和正常腹膜组织的单细胞转录组图谱

通过对 29 例原发 GC 组织、11 例正常胃组织、4 例胃癌腹膜转移灶组织和 1 例 GC 患者正常腹膜组织进行 scRNA-seq 分析，鉴定了 8 种主要的细胞群，包括 T 细胞（T cells）、上皮细胞（Epithelial cells）、髓系细胞（Myeloid cells）、内皮细胞（Endothelial cells）、B 细胞（B cells）、成纤维细胞（Fibroblasts）、周细胞（Pericytes）和肥大细胞（Mast cells）。

通过比较 8 种细胞在不同组织中的浸润数量和浸润深度，结果显示：T 细胞总体浸润的数量最多，其次为上皮细胞和 B 细胞，周细胞总体浸润的数量最少；T 细胞在原发 GC 和胃癌腹膜转移灶中浸润深度最高，周细胞在原发 GC 中浸润最低；肥大细胞在胃癌腹膜转移灶中浸润最低。

通过 HALLMARK 基因集功能富集分析发现，在 GC 转移癌中，8 种细胞群的功能主要集中在细胞增殖、细胞代谢、细胞免疫、DNA 损伤、细胞发生发展和构成细胞组分等方面，而成纤维细胞与细胞侵袭和迁移相关的 EMT 通路关系最密切。

通过评估 GC TME 中 8 种细胞浸润程度与 GC 患者总生存期的相关性，结果显示在 8 种细胞中，只有成纤维细胞和周细胞的浸润程度与 GC 患者不良预后呈负相关。

CD248+成纤维细胞与晚期 GC 的侵袭和转移密切相关

2.1 提取上述 scRNA-seq 结果中的成纤维细胞亚群，并进一步重新降维聚类，鉴定出 11 个成纤维细胞亚群，分别依次命名 Fib1-11。

2.2 Fib6（CD248+成纤维细胞）高表达膜蛋白 CD248 和表达 ECM 有关的部分基因（除了 POSTN），但不表达 ACTA2 等经典活化成纤维细胞的标记物，有别于其他经典的 CAFs，例如 mCAFs，apCAFs 和 iCAF 等，即命名为 CD248+成纤维细胞。

2.3 比较了 11 种成纤维细胞亚群在不同组织中的浸润数量和浸润深度，结果显示：在原发 GC 中，Fib1 的浸润数量最多，Fib8 的浸润数量最少；而在胃癌腹膜转移灶中，Fib6（CD248+成纤维细胞）的浸润数量最多，Fib4 最少；Fib1、Fib10 和 Fib11 只存在于原发 GC 和正常胃组织中。Fib4 在原发 GC 中的浸润深度最高，Fib11 的浸润深度最低；Fib6（CD248+成纤维细胞）在 GC 转移癌中的浸润深度最高，Fib4 的浸润深度最低。

2.3 采用 HALLMARK 基因集富集 Fib6（CD248+成纤维细胞）的功能，发现：Fib6（CD248+成纤维细胞）高度富集上皮-间叶细胞转化（Epithelial–mesenchymal transition, EMT）通路和 COAGULATION 通路；GO 富集分析中，Fib6（CD248+成纤维细胞）胞内大量 ECM 相关的通路和 BMP 通路激活。

2.4 分析了 Fib6（CD248+成纤维细胞）在 GEO 数据库中的表达与临床特征和病理意义之间的关系，结果显示：Fib6（CD248+成纤维细胞）在晚期 GC 患者中的浸润程度高，在“EMT”病理分型的 GC 患者中的浸润程度高于其他分型的 GC 患者，此外，Fib6（CD248+成纤维细胞）浸润程度与 GC 患者的不良预后呈负相关。

CD248+成纤维细胞促进胃癌细胞的侵袭和迁移

3.1 分离、培养和鉴定成纤维细胞并使用慢病毒构建 CD248+成纤维细胞和 CD248-成纤维细胞。

3.2 Transwell 迁移实验分析分别经 CD248+成纤维细胞和 CD248-成纤维细胞共培养后的胃癌细胞（Gastric cancer cells, GCC）与对照无细胞共培养且仅添加 DMEM 培养后的 GCC 的迁移能力，结果显示：CD248+成纤维细胞显著促进 GCC 迁移。

3.3 Matrigel-Transwell 侵袭实验分析分别经 CD248+成纤维细胞和 CD248-成纤维细胞共培养后的 GCC 与对照无细胞共培养且仅添加 DMEM 培养后的 GCC 的侵袭能力，结果显示：CD248+成纤维细胞显著促进 GCC 侵袭。

四、结论

1. 对 GEO 数据库中 29 例原发 GC 组织、11 例正常胃组织、4 例胃癌腹膜转移灶组织和 1 例 GC 患者正常腹膜组织的 scRNA-seq 数据进行了分析挖掘。

2.鉴定了 8 种主要的细胞群及在上述 4 种组织中的浸润情况，T 细胞在四种组织中浸润的数量最多，周细胞浸润的数量最少；T 细胞在原发 GC 和转移癌组织中浸润深度最高，周细胞在原发 GC 组织中浸润深度最低，而肥大细胞在转移癌组织中浸润深度最低。

3.HALLMARK 基因集功能富集分析发现，相比于其它 7 种细胞，成纤维细胞高度富集与细胞侵袭和转移相关的 EMT 通路。

4.鉴定了 11 个成纤维细胞亚群，其中，Fib6（CD248+成纤维细胞）亚群高表达膜蛋白 CD248，定义为 CD248+成纤维细胞，区别于其他亚群。

5. CD248+成纤维细胞亚群高度富集 EMT 通路，在胃癌腹膜转移灶和晚期 GC 中浸润程度高，且与患者预后呈负相关关系。

6.体外迁移和侵袭实验证实 CD248+成纤维细胞亚群能促进胃癌细胞的迁移和侵袭。

因此，CD248+成纤维细胞亚群有可能成为针对 GC 侵袭转移治疗的新靶标。

【关键字】胃癌（GC）；侵袭和转移；成纤维细胞；单细胞转录组

胶质母细胞瘤中缺氧巨噬细胞的鉴定及其对血管正常化的治疗潜力

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【摘要】恶性胶质瘤抵抗常规治疗，复发率高，患者生存期短。胶质瘤内大量浸润的肿瘤相关巨噬细胞（Tumor-associated macrophage, TAM）易在肿瘤和微环境因素作用下发生差异性表型极化。但胶质瘤内 TAM 空间表型异质性特征及其形成机理尚不清楚。TAM 在胶质瘤微血管周围富集，但其如何参与胶质瘤微血管结构重塑及其临床诊疗意义尚不清楚。

我们分析了 51 例成人胶质瘤的单细胞转录组，绘制单核细胞来源和小胶质细胞来源 TAM 的单细胞图谱，鉴定出富集缺氧反应等六个新功能亚群并验证其功能；建立分析胶质瘤 bulk 转录组中 TAM 各亚群含量的模型，阐明 TAM 亚群构成与胶质瘤类型、级别、分子变异和患者预后的相关性；利用空间转录组绘制 TAM 各亚群的空间图谱，明确其在血管富集区、缺氧坏死区和侵袭前沿区的空间分布差异；聚焦缺氧坏死区的低氧和肿瘤因素，阐明其诱导 TAM 缺氧表型极化的机制。此外，胶质瘤血管周 TAM 旁分泌 ADM 是破坏微血管内皮间连接而导致微血管高渗漏和低灌注的重要原因；靶向清除 ADM+ TAM 或阻断 ADM 旁分泌轴能诱导微血管结构正常化，提高抗肿瘤药物递送效率。

【关键字】胶质瘤、肿瘤相关巨噬细胞、单细胞/空间转录组、缺氧、血管正常化

PTEN 棕榈酰化调控增强胶质瘤化疗药敏感性的机制研究

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【摘要】胶质瘤是最常见的颅内恶性肿瘤，胶质瘤具备手术难以切除、放化疗效果差、易复发和预后差的特点，但放射治疗还是胶质瘤最有效的治疗手段之一，因此增强放化疗药敏感性是现在的研究热点。PTEN 是一种肿瘤抑制基因，其编码的具有磷酸酯酶活性的蛋白，是机体维持正常生理功能所必须的因子。PTEN 可通过多种通路影响细胞增殖、代谢，进而抑制肿瘤的发生发展。棕榈酰化是一种蛋白质的翻译后修饰机制，其中棕榈酸基通过硫酯键（也称为 S-棕榈酰化）与绝大多数的半胱氨酸残基共价连接。通过影响蛋白质膜的锚定，运输，相互作用和降解，棕榈酰化在包括癌症在内的人类生理和病理过程中起着重要作用。几种癌症相关的蛋白，如 EZH2，TEAD 和 c-Met，被棕榈酰化用于稳定，敲低 ZDHHC5，EZH2 的棕榈酰转移酶，显著抑制神经胶质瘤肿瘤的生长。然而 PTEN 棕榈酰化在胶质瘤的作用目前尚不清楚。因此研究 PTEN 棕榈酰化在胶质瘤中的作用和机制对于进一步阐释胶质瘤的发病机制以及对于胶质瘤的治疗具有重要的临床意义。

我们的初步研究发现 PTEN 在胶质瘤细胞系中高表达并且具有显著的棕榈酰化修饰，棕榈酰化抑制剂 2-溴棕榈酸酯（2-BP）的处理显著下调 PTEN 的蛋白表达水平及其棕榈酰化水平，说明 PTEN 的棕榈酰化影响其稳定性。棕榈酰化抑制剂处理或突变 PTEN 的棕榈酰化位点不仅影响了其稳定性，还影响了 PTEN 的泛素化及其与泛素连接酶 E3 的结合。初步的体外实验发现，棕榈酰化抑制剂能增强胶质瘤化疗药的敏感性。初步的小鼠动物实验发现，棕榈酰化抑制剂或突变 PTEN 棕榈酰化位点显著增强胶质瘤化疗药的敏感性。因此，我们推测 PTEN 通过棕榈酰化作用影响其稳定性进而增强胶质瘤化疗药的敏感性，靶向 PTEN 的棕榈酰化修饰可能是提高胶质瘤治疗的新策略。

【关键字】PTEN；棕榈酰化；稳定性；泛素化；胶质瘤

CD47 乙酰化调控在胶质瘤免疫逃逸中的作用研究

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【摘要】胶质瘤是最常见的颅内恶性肿瘤，胶质瘤具备手术难以切除、放化疗效果差、易复发和预后差的特点，随着肿瘤免疫治疗技术的进步与发展，给胶质瘤的治疗带来了新的希望。CD47 是癌细胞广泛表达的重要免疫检查点，其作为一种“不要吃我”的信号分子，与巨噬细胞表面 SIRPα相互作用，从而使肿瘤细胞避免被吞噬清除，增强肿瘤生存能力。乙酰化修饰作为常见的翻译后修饰机制，通过多种机制影响蛋白质功能，包括调节蛋白质稳定性，酶活性，亚细胞定位和与其他翻译后修饰的 crosstalk 以及通过调控蛋白质-蛋白质、蛋白质-DNA 相互作用等。然而 CD47 乙酰化在胶质瘤的作用目前尚不清楚。因此研究 CD47 乙酰化在胶质瘤中的作用和机制对于进一步阐释胶质瘤的发病机制以及对于胶质瘤的免疫治疗具有重要的临床意义。

我们的初步研究发现 CD47 在胶质瘤细胞系 U87、LN18 中高表达并且具有显著的乙酰化修饰，而乙酰化酶抑制剂的处理显著下调 CD47 的乙酰化水平。免疫共沉淀结果显示，突变 CD47 乙酰化位点或乙酰化酶抑制剂的处理显著减弱 CD47 与其受体 SIRPα的结合。体外吞噬实验显示，突变 CD47 乙酰化位点或乙酰化酶抑制剂的处理显著促进巨噬细胞 THP1 对胶质瘤细胞 U87 及 LN18 的吞噬作用。初步的小鼠动物模型实验发现，突变 CD47 乙酰化位点显著抑制胶质瘤细胞 U87 及 LN18 的肿瘤生长。因此，我们推测 CD47 通过乙酰化作用增强与其受体 SIRPα的结合进而促进胶质瘤细胞的免疫逃逸，靶向 CD47 的乙酰化修饰可能是胶质瘤免疫治疗的新策略。

【关键字】CD47；乙酰化；CD47/SIRPα 信号通路；胶质瘤；免疫逃逸

FANCD2 缺失通过铁死亡增加 SHH 亚型髓母细胞瘤对放疗的敏感性

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【摘要】放射治疗是髓母细胞瘤（MB）标准治疗方案之一。肿瘤细胞利用 DNA 损伤修复（DDR）机制在放疗过程中产生抵抗性。研究发现，针对 DDR 的靶向治疗可以增强多种肿瘤细胞对放疗的敏感性，但 DDR 在 MB 放疗反应中的作用及机制尚待明确。本研究对 4 例 MB 组织进行了单细胞转录组测序，同时结合 34 例数据库中 MB 的单细胞数据进行生信分析。通过评估 MB 样本和公共 MB 数据库中的 FANCD2 表达，利用细胞增殖试验（CCK-8）、克隆形成试验、乳酸脱氢酶试验和小鼠原位脑肿瘤模型探讨了 FANCD2 在 MB 细胞中的功能。随后，通过脂质过氧化检测、丙二醛(MDA)检测、还原型谷胱甘肽检测以及 FerroOrange 评估细胞内亚铁离子（Fe^{2+}）含量来探讨 FANCD2 相关的信号通路。研究结果显示，FANCD2 在 SHH 亚型的 MB（SHH-MB）中高表达。FANCD2 在 SHH-MB 患者中起癌基因的作用，同时预示着较差的预后。FANCD2 的敲低显著抑制了 SHH-MB 细胞的增殖、迁移，并增强了 SHH-MB 细胞对放疗的敏感性。从机制上讲，FANCD2 缺失导致 Fe^{2+}的堆积，主要是由于 DMT1 表达的增加和 GPX4 活性的减弱，进一步诱导了铁死亡。通过使用原位小鼠脑肿瘤模型，我们观察到放疗结合 FANCD2 的敲低显著抑制了体内 SHH-MB 细胞来源肿瘤的生长。我们的研究揭示了 FANCD2 可作为 SHH-MB 的潜在治疗靶点，敲低 FANCD2 可以通过诱导铁死亡来增强 SHH-MB 对放疗的敏感性。

KPT330 通过保留细胞核中的 SQSTM1 和破坏溶酶体功能来促进胶质母细胞瘤对奥拉帕尼的敏感性

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【摘要】Poly (ADP-ribose) polymerase (PARP) inhibitors have demonstrated promising clinical activity in multiple homologous recombination (HR) deficiency tumors. However, glioblastoma (GBM) patients have obtained little benefit from PARP inhibitors alone. PARP inhibition shows considerable promise when used together with other therapeutic agents. Thus, novel combination therapies may enhance PARP inhibitor efficacy and overcome resistance mechanisms in GBM. Herein, we report that concurrent treatment with the PARP inhibitor olaparib and Exportin 1 (XPO1) inhibitor KPT330 showed synergetic anticancer effects on GBM cells. Mechanistically, in the nucleus, we show that KPT330 induced the nuclear retention of Sequestosome 1 (SQSTM1) and further inhibited the ubiquitination of the DNA repair signal H2A.X Variant Histone (H2AX) mediated by olaparib, thus inhibiting DNA damage response and repair in GBM. Moreover, in the cytoplasm, KPT330 blocked the activation of autophagic flux caused by olaparib reagent, downregulated the expression of lysosomal-associated transmembrane protein 4B (LAPTM4B) and induced the dysfunction of lysosomes, thereby preventing the degradation of autophagosome, and ultimately promoted cell death. Furthermore, in the LN229-luc mouse orthotopic xenograft model, combination treatment showed significantly increased antitumor efficacy compared to each monotherapy. These data illustrate the application prospects of combined oral administration of olaparib and KPT330 for the treatment of glioblastoma.

【关键字】Glioblastoma; Olaparib; KPT330; SQSTM1; Autophagic flux; Lysosome

滤泡树突状细胞（FDC）分泌 Ntn1 调控生发中心（GC）反应稳态

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【摘要】滤泡树突状细胞（FDC）通过释放 Cxcl13 招募 B 细胞并向 B 细胞提供抗原免疫复合物，在生发中心（GC）的形成中发挥关键作用，其功能异常伴随着 GC 不能正常形成。Tfh 细胞是定位于 GC 亮区，对于 GC 形成至关重要的另一类重要细胞，虽然 Tfh 细胞与 FDC 细胞共同定位于生发中心亮区，但目前领域内尚不清楚这两种细胞是否存在相互作用。我们前期研究发现，FDC 特异性分泌神经导向因子 Ntn1，FDC 细胞特异性敲除 Ntn1 导致 GC 反应和特异性抗体生成减弱。进一步我们发现 Ntn1 的受体 unc5a 高表达于 Tfh 细胞，这提示我们来源于 FDC 的 Ntn1 可能对 Tfh 细胞的功能有重要影响。因此，我们将结合基因改造小鼠，单细胞等手段，和 BCR 测序等方法，深入研究：1.FDC 细胞通过分泌 Ntn1 对 Tfh 细胞分化分布的影响；2.FDC 细胞通过分泌 Ntn1 对生发中心反应以及抗体生成的影响。

线粒体丙酮酸氧化对始发态人胚胎干细胞的生存至关重要

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【摘要】Human embryonic stem cells (hESCs) are derived from the inner cell mass (ICM) of pre-implantation blastocysts and have the capacity of unlimited self-renewal and developmental pluripotency in vitro. hESCs at different pluripotency states, including naïve, formative, and primed, represent the in vivo counterpart pluripotent cells at the developmental stage of pre-, peri-, or post- implantation, respectively. Thus, hESCs serve as an ideal in vitro model to unveil the underlying mechanisms of human early embryo development. It is clear that metabolic patterns are closely related to early embryo development stages. However, the question of how metabolism determines the cell fate remains largely unsolved. Given the critical role of pyruvate in mammalian pre-implantation embryos, herein, we attempted to figure out how mitochondrial pyruvate oxidation affects cell fate determination using hESCs at different pluripotency states. To this end, we employed a genetically inducible knock-out hESC line for DLAT, encoding a critical subunit of the pyruvate dehydrogenase complex (PDHc), and a pharmacologic inhibitor of the mitochondrial pyruvate carrier (MPC). Notably, DLAT ablation or MPC inhibition led to a reduction of acetyl-CoA abundance and histone acetylation levels as well as cell death and disrupted transcriptional programs in primed hESCs. Unexpectedly, DLAT ablation elicited little impact on cell survival and histone acetylation in naïve hESCs. Intriguingly, long-term DLAT inhibition or MPC suppression reduced the GATA3 positive and NANOG negative subpopulation of naïve hESCs. These findings suggest that mitochondrial pyruvate oxidation is vital for primed pluripotency but not for naïve pluripotency. Our study underscores the relationship between pyruvate metabolism and cell fate determination in human early embryo development at post-implantation stages.

【关键字】hESC, DLAT, pyruvate metabolism, histone acetylation, pluripotency state

乙酰转移酶 HBO1 促进卵巢癌上皮间质转化和免疫逃逸

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【摘要】高级别浆性卵巢癌是妇科最常见的癌症之一，死亡率高，常规的治疗方案是手术切除和化疗相结合，但是难以控制其进展并且容易复发。上皮间质转化（EMT）能促进肿瘤细胞迁移侵袭，增加其耐药和免疫逃逸的能力，但是肿瘤细胞发生 EMT 的具体机制还有待研究。我们发现在高级别浆性卵巢癌中乙酰转移酶 HBO1 是驱动细胞内 EMT 的决定性因素。通过 TGFβ细胞因子诱导卵巢癌发生 EMT，伴随着 HBO1 的过表达；而抑制 TGFβ信号通路削弱了卵巢癌细胞的迁移能力，导致 HBO1 的表达显著降低。在卵巢癌细胞中敲降 HBO1，会阻碍 TGFβ驱动的 EMT 发生，削弱肿瘤细胞的迁移、侵袭以及在体内形成肿瘤的能力。有趣的是，缺失 HBO1 的卵巢癌细胞其 MHC I 类分子表达增加，而免疫检查点受体 PD-L1 的表达减少，这提高了肿瘤细胞对 CAR-T 细胞治疗的敏感性，显著地减少了 T 细胞的耗竭。机制上，在卵巢癌细胞中 HBO1 和 SMAD4 共同定位在 EMT 相关的转录因子以及转移和代谢等相关基因的启动子上，促进并维持这些基因的表达。综上所述，我们发现乙酰转移酶 HBO1 在卵巢癌进展中的关键作用，揭示了其介导的表观遗传调控机制，为革新卵巢癌的治疗方案提供了新的思路。

【关键字】卵巢癌、乙酰转移酶 HBO1、TGFβ通路、上皮间质转化、免疫逃逸

核糖调控控制 FXR1 凝聚体以协调 mRNA 的本地化翻译和转运

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【摘要】Biomolecular condensates are found throughout a diversity of eukaryotic cell types and cellular compartments and are involved in a variety of cellular functions. A given protein generally forms functionally and compositionally heterogeneous condensates, but the underlying regulatory mechanisms are unknown. Here, we found that different RNA motifs can modulate the formation of heterogeneous mRNA-protein complex (mRNP) condensates via riboregulation. Fragile X-related 1 (FXR1), identified as an mRNA export acceptor, assembles distinct localized subcellular mRNP condensates that facilitate the nuclear export of G-quadruplex-containing pluripotent mRNAs and mediate the localized translation of nucleoporin mRNAs at nuclear pores. The diverse locations and functions of FXR1 condensates depend on the unique RNA-protein interaction modules of its two RNA binding domains, as well as the opposing effects of binding different RNA motifs on the affinity of FXR1 for nuclear pores. More importantly, a decline in FXR1 and nuclear pore activity hinders the nuclear export of transcribed RNA, thereby aiding human embryonic stem cells (hESCs) achieving fate transition. Preventing this decline would result in impaired hESC differentiation.

【关键字】 biomolecular condensates, FXR1, mRNA fate, hESC differentiation

利用单细胞转录组技术解析小鼠全能干细胞的异质性

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【摘要】全能干细胞可以形成胚胎和胚外组织，具有强大发育潜能的一种细胞类型。在小鼠中，只有受精卵至合子激活期的二细胞胚胎具有全能性。在体外获得类似小鼠胚胎 2 细胞卵裂球、可以长期稳定培养的全能干细胞一直是干细胞研究领域的一个重大挑战。我们前期研究，通过重塑着丝粒周围的异染色质和重建全能性特异性宽 H3K4me3 结构域，获得了一种类似小鼠 2 细胞卵裂球、可以在体外稳定培养的全能干细胞，且具有向胚胎和胚外双向发育的能力。然而体外诱导获得的全能干细胞存在较大的异质性，阻碍了全能干细胞作为体外研究胚胎发育模型的应用。本研究通过对比小鼠早期胚胎卵裂球以及近期发表的各种小鼠全能干细胞（TLSC、TBLC、TPSC 和 TotiSCs）的单细胞转录组数据，发现小鼠全能干细胞的异质性与其培养条件有关，特定培养条件通过激活视黄酸信号通路等来诱导胚外谱系细胞的产生。基于上述发现，我们对小鼠全能干细胞的培养体系进行优化，显著降低了小鼠全能干细胞培养中的胚外谱系细胞比例。该研究为全能干细胞更深入的理解和利用类囊胚模型研究早期胚胎发育提供了一种更为可靠的细胞模型。

【关键字】全能干细胞；单细胞转录组；异质性

人类固有淋巴细胞的初始起源解析

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【摘要】Hematopoietic stem cell (HSC)-independent lymphopoiesis has been elucidated in murine embryos. However, our understanding regarding human embryonic counterparts remains limited. Here, we unveiled the presence of human yolk sac-derived lymphoid-biased progenitors (YSLPs) expressing CD34, IL7R, LTB and IRF8 at Carnegie Stage 10, much earlier than the first HSCs emergence. The number and lymphopoietic potential of these progenitors were both significantly higher in yolk sac than embryo proper at this early stage. Importantly, single-cell/bulk culture and CITE-seq have elucidated the tendency of YSLP to differentiate into innate lymphoid cells and dendritic cells. Notably, lymphoid progenitors in fetal liver before and after HSC seeding displayed distinct transcriptional features, with the former closely resembling those of YSLPs. Overall, our data deciphered the origin, potential, and migratory dynamics of innate lymphoid-biased multipotent progenitors in human yolk sac before HSCs emergence, providing insights for understanding the stepwise establishment of innate immune system in humans.

【关键字】innate lymphoid cell; yolk sac; lymphopoiesis; hematopoietic stem cell; HSC-independent; hematopoiesis; natural killer cell; dendritic cell; human; embryonic

三、干细胞转化研究

人脑类器官移植到嗅球中的气味响应

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【摘要】At present, brain organoids generated from human pluripotent stem cells have made breakthroughs in the study of neural development, pathogenesis of nervous system diseases and other aspects. There have been studies that applied human brain organoids to the treatment of neurological diseases with massive injuries such as traumatic brain injury and stroke. However, the repair of olfactory damage caused by neurological diseases such as Parkinson ‘s disease and Alzheimer’ s disease still needs further exploration. Here, we demonstrate that human cortical brain organoids can successfully form connections with the host tissue after transplantation into the mouse olfactory bulb. When the mice are stimulated by odors, response of neurons in olfactory bulb organoids can be recorded by flexible electrodes and different odors demonstrate different response patterns. These results provide experimental support for future clinical transplantation of stem cells to treat olfactory diseases.

干细胞治疗帕金森病可行性分析

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【摘要】帕金森病（PD）是一种常见的神经退行性疾病，尽管目前的药物和手术治疗能够显著提高帕金森病患者的生活质量，但其只能短期的缓解相应症状，尚不能治愈或者延缓帕金森病的进程。由于帕金森病经典的运动症状很大程度上是中脑黑质多巴胺能神经元的变性死亡导致的，通过干细胞移植补充多巴胺能神经元，从而治疗帕金森病一直以来被认为具有其巨大的临床应用前景。基于对近年来干细胞移植治疗帕金森病的研究进展的归纳总结，本文就干细胞治疗帕金森的可行性以及存在的利弊展开讨论。

【关键字】干细胞移植；神经再生；帕金森病；多巴胺能神经元

间充质干细胞抑制下丘脑 CRH 神经元兴奋改善卒中后免疫缺陷的机制研究

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【摘要】背景：急性缺血性卒中（AIS）后下丘脑-垂体-肾上腺轴（HPA）轴异常激活引发糖皮质激素水平上升，是淋巴细胞减少、脾脏萎缩和免疫功能缺陷的主要原因，会增加重症患者的感染风险和死亡率。然而，目前的干预措施未能改善这些患者的临床结局。间充质干细胞（MSC）在急性缺血性卒中临床治疗中都体现了良好的治疗效果，我们之前的研究表明 MSC 通过保护边缘区 B 细胞（MZB）改善 AIS 小鼠的自发感染，可作为 AIS 后免疫缺陷的潜在解决方案。

目的：MSC 是否通过调控 HPA 轴改善卒中后免疫功能缺陷未知。

结果：在本研究中，我们发现 MSC 治疗能显著降低大脑中动脉栓塞模型（MCAO）小鼠外周血和腹腔液中的菌载量，改善卒中后继发感染症状。接下来，我们发现 MSC 能降低 MCAO 小鼠外周血和脾脏糖皮质激素水平，改善 MCAO 小鼠脾脏基质细胞和淋巴细胞的死亡。为了评估 MSC 治疗效果是否与 HPA 轴相关，我们检测了 HPA 轴相关激素，发现除糖皮质激素外，静脉注射的 MSC 还能显著降低促肾上腺皮质激素释放激素（CRH）/促肾上腺皮质激素（ACTH）的水平，这可能是由于 MSC 对卒中诱导的下丘脑室旁核 CRH（CRH PVN）神经元异常激活的抑制作用。最后，我们发现 MSC 可以跨越 MCAO 小鼠受损的血脑屏障进入中枢，通过提高卒中后小鼠中枢抗氧化能力，改善氧化应激，抑制卒中后 CRH PVN 神经元的过度应激反应，调控 HPA 轴缓解卒中后小鼠的免疫功能缺陷。

结论：静脉输注 MSC 通过抑制 CRH PVN 神经元的过度氧化应激，调控 HPA 轴的异常活化，从而改善卒中后小鼠的脾脏萎缩和免疫功能缺陷。

人骨髓间充质干细胞静脉给药在孤独症治疗中的作用机制及转化研究

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【摘要】目的：孤独症谱系障碍（ASD）是一类发生于儿童早期的神经发育障碍性疾病，目前尚无针对孤独症的有效药物。间充质干细胞（MSC）因其具备低免疫原性、免疫调节能力、多潜能分化能力等特征成为拥有广泛前景的新型治疗药物。在本研究中，我们通过连续观测 BTBR 孤独症模型小鼠（BTBR 鼠）行为学探讨了人骨髓来源间充质干细胞（hBMMSC）静脉给药对 ASD 核心症状的治疗作用并首次阐明 hBMMSC 对 BTBR 鼠肠道菌群具有调节作用。

材料和方法：使用 BTBR 小鼠作为 ASD 模型，随机分配至 hBMMSC 静脉注射治疗组或对照组。进行为期 6 周的多项社交行为测试，并在不同时间点收集粪便样本进行 16S rRNA 测序分析。

结果：hBMMSC 治疗改善了 BTBR 小鼠在旷场试验、明暗箱试验、新物体识别试验和自由社交试验中的行为缺陷，并显著减少了小鼠的刻板行为。hBMMSC 治疗显著逆转了 BTBR 小鼠肠道菌群丰度的变化，特别是拟杆菌门/厚壁菌门比率。hBMMSC 治疗改变的差异菌属相对丰度与 BTBR 鼠行为学相关性极高。

结论：本研究表明 hBMMSC 静脉给药可以纠正 BTBR 孤独症模型小鼠的核心行为学症状。本研究进一步证明了 hBMMSC 静脉给药可影响孤独症小鼠肠道菌群，对孤独症治疗产生积极影响。

【关键字】孤独症谱系障碍，人骨髓间充质干细胞，肠道菌群

缺血性脑卒中的神经干细胞治疗及作用机制

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【摘要】缺血性脑卒中是一种由于脑血管狭窄或堵塞导致脑组织死亡的脑部疾病，具有高发病率、高致残率、高死亡率的特点。脑卒中会引发严重的兴奋性毒性和神经炎症，从而导致神经元死亡，神经血管单元损伤、血脑屏障破坏以及脑水肿的发生。目前针对缺血性脑卒中的有效干预措施依然是再灌注治疗，包括溶栓和机械取栓，但这种方法受到严格的时间窗限制，且无法改变由于神经元死亡导致的病理结局。神经干细胞可以分化为神经元，也可以通过旁分泌作用分泌营养因子，从而减轻神经炎症，因此近年来神经干细胞在脑卒中治疗中展现了极大的潜力。然而，神经干细胞治疗存在标准化质控困难和分化命运不可控等问题，削弱了其治疗效果。因此，我们的研究聚焦于寻找和开发适合体内移植和临床治疗的神经干细胞分化方法，并验证其在病理环境中的命运和治疗效果，以及探究其作用机制。首先，我们筛选多种目前广泛应用于移植的神经干细胞分化方法，找到了分化稳定、质量均一，适合临床治疗的分化方法，并建立了标准化质控体系。第二，将神经干细胞移植入缺血性脑卒中小鼠体内后，高比例分化为神经元，极少分化为星形胶质细胞，不分化为少突胶质细胞，此外，我们还探究了命运偏向的可能原因。第三，除了直接的神经元补充作用之外，我们发现神经干细胞还能够促进宿主神经再生和血管功能恢复，促进了宿主运动功能恢复，减少了脑梗死体积，针对性的解决了缺血性脑卒中治疗的棘手问题，为进一步的临床实验打下坚实基础，极大推动了缺血性脑卒中的细胞治疗进程。

【关键字】缺血性脑卒中；细胞治疗；神经干细胞；神经发生；血管重塑

Mechanical priming regulates fibrotic mechanical memory of mesenchymal stem cell through YAP in spinal cord injury repair

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【摘要】Spinal cord injury (SCI) is a public health problem with high morbidity and causes severe economic burden. Recently, mesenchymal stem cell (MSC) has a broad clinical application prospect in treating SCI. However, the transplantation efficacy of MSC for SCI, especially in the chronic stage, is unstable. Hence, further exploration of the key factors limiting the effectiveness of MSC transplantation for SCI is urgently needed. Here, we found that the dominant microenvironmental change in the injured spinal cord is the elevated matrix stiffness due to excessive secretion of extracellular matrix in the scar-centered environment. Transplanted MSC colonized in the center of the scar for at least 2 weeks and demonstrated fibrotic phenotypic transformation. Further, we cultured MSC on polyacrylamide gels with different matrix stiffness in vitro and found that elevated matrix stiffness is the determining factor to the increase of fibrotic phenotype and function and the decrease of immunomodulatory capacity in MSC, leading to reduced therapeutic function. Mechanistically, the classic mechanical effector YAP was accumulated and activated in the nucleus, which contributed to the consequent pro-fibrotic cell programs activation in MSC. Furthermore, considering that MSC has mechanical memory, which demonstrates that cells permanently imprint information regarding substrate mechanical conditions, thus, to improve the therapeutic effect of MSC, we conducted long-term soft priming of MSC before transplantation. Such soft-primed MSC could inhibit the nuclear distribution and activation of YAP, protecting MSC against mechanical activation with low fibrogenic character and high immunomodulatory capacity under stiff substrates. Finally, we found that MSC priming on physiologically soft substrates when transplanted to an animal model of SCI could promote SCI repair, including significant improvement in inflammation, fibrosis, and motor function both histologically and functionally. In conclusion, this study systematically elucidated the effects of matrix stiffness and mechanical priming on the fate of transplanted MSC from a new perspective of biomechanics, which provides a scientific basis and theoretical foundation for optimizing the treatment of MSC therapy and improving its efficacy in spinal cord injury repair.

【关键字】Spinal cord injury; Mesenchymal stem cell; Mechanical priming; Fibrotic mechanical memory; YAP

Precise Correction of Lhcgr Mutation in Stem Leydig Cells by Prime Editing Rescues Hereditary Primary Hypogonadism in Mice

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【摘要】Hereditary primary hypogonadism (HPH), caused by gene mutation related to testosterone synthesis in Leydig cells, usually impairs male sexual development and spermatogenesis. Genetically corrected stem Leydig cells (SLCs) transplantation may provide a new approach for treating HPH. Here, a novel nonsense-point-mutation mouse model (LhcgrW495X) is first generated based on a gene mutation relative to HPH patients. To verify the efficacy and feasibility of SLCs transplantation in treating HPH, wild-type SLCs are transplanted into LhcgrW495X mice, in which SLCs obviously rescue HPH phenotypes. Through comparing several editing strategies, optimized PE2 protein (PEmax) system is identified as an efficient and precise approach to correct the pathogenic point mutation in Lhcgr. Furthermore, delivering intein-split PEmax system via lentivirus successfully corrects the mutation in SLCs from LhcgrW495X mice ex vivo. Gene-corrected SLCs from LhcgrW495X mice exert ability to differentiate into functional Leydig cells in vitro. Notably, the transplantation of gene-corrected SLCs effectively regenerates Leydig cells, recovers testosterone production, restarts sexual development, rescues spermatogenesis, and produces fertile offspring in LhcgrW495X mice. Altogether, these results suggest that PE-based gene editing in SLCs ex vivo is a promising strategy for HPH therapy and is potentially leveraged to address more hereditary diseases in reproductive system.

【关键字】 hypogonadism; prime editing; spermatogenesis; stem Leydig cells; testis; testosterone.

造血干祖细胞膜仿生囊泡用于靶向骨髓递送药物抑制白血病发生

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【摘要】Leukemia is a kind of hematological malignancy originating from bone marrow, which provides essential signals for initiation, progression, and recurrence of leukemia. However, how to specifically deliver drugs to the bone marrow remains elusive. Here, we develop biomimetic vesicles by infusing hematopoietic stem and progenitor cell (HSPC) membrane with liposomes (HSPC liposomes), which migrate to the bone marrow of leukemic mice via hyaluronic acid-CD44 axis. Moreover, the biomimetic vesicles exhibit superior binding affinity to leukemia cells through intercellular cell adhesion molecule-1 (ICAM-1)/integrin β2 (ITGB2) interaction. Further experiments validate that the vesicles carrying chemotherapy drug cytarabine (Ara-C@HSPC-Lipo) markedly inhibit proliferation, induce apoptosis and differentiation of leukemia cells, and decrease number of leukemia stem cells. Mechanically, RNA-seq reveals that Ara-C@HSPC-Lipo treatment induces apoptosis and differentiation and inhibits the oncogenic pathways. Finally, we verify that HSPC liposomes are safe in mice. This study provides a method for targeting bone marrow and treating leukemia.

【关键字】Bone marrow; Targeted delivery; HSPC; Biomimetic vesicles; leukemia

双阴性 T 细胞治疗 5×FAD 阿尔兹海默症小鼠

谢苑芷、刘京、侯宗仁、刘凯伦、李灿、詹泊

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【摘要】Alzheimer's disease is a progressive and irreversible neurodegenerative disorder that affects millions of people worldwide. We investigated whether double-negative T (DNT) cell transplantation could improve AD pathology and associated cognitive deficits in 5×FAD mice. Peripheral administration of DNT cells improved cognitive deficits, enhanced synaptic plasticity and neuron complexity, decreased plaque deposition, and shifted the microglial state toward a neuron-related phenotype. DNT inhibited neuroinflammation and had immune-protective effects. These findings suggest that DNT cell transplantation may be a potential therapy for AD.

多功能干细胞分化来源的间充质干细胞用于急性肝损伤的治疗研究

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【摘要】已有临床试验证实从成体组织中分离培养的间充质干细胞（Mesenchymal stem cells, MSC）能改善患者的肝损伤情况，但此类 MSC 却存在增殖能力弱、易老化、细胞组成不均一及批次差异等缺陷。通过多功能干细胞（Pluripotent stem cells, PSC）体外诱导分化 PSC 来源的 MSC（PSC-MSC）能有效规避上述问题，为临床应用提供更优细胞来源。尽管当前已有一些 PSC-MSC 分化方法的报道，但其分化配方及流程均相对复杂，增加了质控难度，不利于临床转化。我们建立了一种新的、简单、高效且稳定的 MSC 分化体系，能在单一培养条件下，于两周内直接将 PSC 诱导分化为高纯度 PSC-MSC。体外功能实验显示，我们分化的 PSC-MSC 比成体组织来源的 MSC 如骨髓来源 MSC（BM-MSC）具有更优细胞传代次数、增殖能力及抗逆性。为进一步评估该 MSC 对肝损伤的治疗潜力，我们分别将 PSC-MSC 及 BM-MSC 移植到四氯化碳诱导的急性肝损伤小鼠模型。其结果显示，与 BM-MSC 相比，我们分化的 PSC-MSC 能更显著降低小鼠肝细胞损伤及肝纤维化程度，并显著提高小鼠的存活率。接下来，我们将继续通过一系列体内外实验来系统评估上述 PSC-MSC 的临床转化潜力，并阐明该细胞在肝损伤保护及肝再生中的具体作用机制。本研究将为推动 PSC-MSC 的临床转化提供强有力的理论支持，并为开拓肝损伤治疗新方法提供新思路。

shRNA 靶向结合慢病毒载体负链 mRNA 进而提高病毒包装滴度的研究

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【摘要】β-地中海贫血是一种隐性单基因遗传病，主要是由于β珠蛋白基因合成缺陷导致α-和β-珠蛋白链比例失衡所致，基因与细胞治疗是根治该疾病的最佳解决方案。慢病毒载体（LVV）已成为临床试验中基因治疗的常下用载体之一。据报道，LVV 介导的临床试验已经成功地治疗了近百例β-地中海贫血病例。这些 LVVs 携带一个反向放置的β-珠蛋白（HBB）基因表达盒，以在病毒 RNA 包装过程中使其携带的内含子得以保存。因此，这些 LVV 往往在其包装过程中产生由其红系特异的启动子驱动的少量负向转录本，并通过与病毒主链互补而降低病毒滴度。为了克服这一问题，我们设计了专门针对由 LVV 内部启动子驱动的负链转录本的 shRNA，从而显著增加了病毒包装滴度。这一研究显示了一个简单且有效的方法来提高慢病毒载体的包装滴度，进而提升β-地中海贫血基因治疗的效果。

【关键字】慢病毒载体；滴度；RNA 干扰；BB305；反向表达盒

NR5A1 促进人诱导多能干细胞定向分化为卵泡膜细胞及其移植治疗研究

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【摘要】卵巢早衰（Premature ovarian failure，POF）是影响女性生育的常见疾病，表现为雌激素水平降低、生育力下降等；POF 病因复杂且发病机制不明，现有的疗法对 POF 的改善仍然有限。体内雌激素的产生有赖于卵泡膜细胞（theca cells，TCs）提供雄激素原料，我们前期研究表明 TCs 的前体卵泡膜干细胞（thecal stem cells，TSCs）自体移植可有效治疗卵巢早衰食蟹猴。此类细胞在体数量有限，难以大量获得，利用诱导多能干细胞（induced pluripotent stem cells，iPSCs）分化为 TCs，有望解决这一问题。

我们发现将 iPSCs 诱导为中胚层祖细胞再分化为 TCs 有助于提高分化效率，但与成熟 TCs 相比尚有一定差距。在此基础上，我们引入了推动类固醇生成的重要转录因子核受体亚家族 5，A 组，成员 1（NR5A1）。与对照组相比，我们发现在诱导为中胚层祖细胞之后的第三天瞬时过表达 NR5A1，其诱导的细胞在 mRNA 水平高表达类固醇合成相关酶标志物（NR5A1、STAR、CYP17A1）及 TCs 相关标志物（INSL3、PTCH2、LHCGR），其中 STAR、CYP17A1、HSD17B、PTCH2 的表达甚至高于阳性对照（人卵泡膜细胞）。更重要的是，该细胞可分泌高水平的脱氢表雄酮和睾酮。

进一步将诱导获得的 TCs 移植入 POF 小鼠体内，与对照组相比，TCs 移植治疗组的雌激素和抗缪勒管激素分别上调了 67.3%和 67.7%，而促卵泡激素水平降低了 28.9%，接近正常水平。治疗组子宫卵巢湿重增加，原始卵泡和生长卵泡比例增加、闭锁卵泡比例减少。这些结果表明 TCs 移植可显著改善 POF 小鼠性激素分泌水平、提升卵巢贮备，为 POF 治疗及保护女性生育力提供了新的治疗策略。

【关键字】诱导多能干细胞；卵泡膜细胞；NR5A1；卵巢早衰

YTHDF2 小分子抑制剂扩增造血干细胞的作用与机制研究

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【摘要】Hematopoietic stem cell transplantation has been widely used in treating hematological malignancies such as leukemia and lymphoma. Although hematopoietic stem cells (HSCs) from umbilical cord blood have lower immunogenicity than adult HSCs, the limited number of HSCs in single unit umbilical cord samples severely restricts its clinical applications. Therefore, it' s the holy grail in the HSC field to efficiently expand HSCs ex vivo. Previous study by our team has shown that the deletion of YTHDF2, a m6A reader protein, significantly accumulates mRNAs related to HSC self-renewal and promotes HSC expansion in both murine and human umbilical cord blood HSCs. Our preliminary work has employed the computerized virtual high-throughput screening technology to identify specific small molecule inhibitors targeting YTHDF2, verified that these inhibitors can enhance HSC ex vivo expansion and in vivo reconstitution capacity. In this project, we will exploit high-throughput sequencing techniques such as ActD-seq and RNA-seq, cellular thermal shift assay (CETSA) and drug affinity responsive target stability (DARTS) assay to demonstrate the mechanisms of YTHDF2 inhibitors in HSC expansion. This project will further our understanding of the roles of m6A modification in maintaining hematopoietic homeostasis and provide new insights into ex vivo expansion and clinical applications of umbilical cord blood HSCs.

间充质干细胞介导的 Bi2Se3 纳米放射增敏剂靶向输送用于非小细胞肺癌的放射治疗

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【摘要】目的：研究间充质干细胞（MSCs）靶向递送纳米放射增敏剂增强非小细胞肺癌（NSCLC）的放射治疗效果。

方法：制备含有高原子序数元素的纳米颗粒硒化铋（Bi2Se3）作为放射增敏剂。利用脂肪来源的间充质干细胞（AD-MSCs）作为载体，通过细胞内装载策略将 Bi2Se3 纳米颗粒装载到 AD-MSCs 中，形成 AD-MSCs/Bi2Se3 复合物。分别在细胞和荷瘤小鼠层次研究了 AD-MSCs/Bi2Se3 复合物对肿瘤细胞的传递效率和放射增敏效果。

结果：AD-MSCs/Bi2Se3 复合物在体外显示出对非小细胞肺癌 A549 细胞的显著放射增敏效果，能够提高 X 射线诱导的 DNA 损伤并抑制肿瘤细胞增殖。在体内，AD-MSCs/Bi2Se3 复合物展现出优异的肿瘤靶向能力，与游离 Bi2Se3 纳米颗粒相比，肿瘤区域的 Bi2Se3 积累增加了 20 倍，且与 X 射线辐照联合使用时，能有效控制肿瘤进展并提高动物模型的生存率。

结论：此复合物利用 AD-MSCs 的固有肿瘤归巢特性，为 NSCLC 的靶向放射治疗提供了一种有前景的策略。

TRIM29 通过泛素化降解 LZTR1 促进胆囊癌吉西他滨耐药的作用及机制研究

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【摘要】吉西他滨的耐药性被认为是导致胆囊癌预后不良的重要因素之一，然而目前对于胆囊癌中吉西他滨耐药的具体机制尚未完全明确。我们前期构建了胆囊癌吉西他滨耐药的胆囊癌 PDX 及细胞模型，RNA-seq 结果表明与肿瘤干细胞相关的基因及 JAK/STAT3 通路在耐药 PDX 及细胞中显著富集。进一步通过功能实验结果筛选到 E3 泛素连接酶 TRIM29 能够促进胆囊癌细胞对吉西他滨的耐药性。机制上，免疫共沉淀结合质谱实验揭示了 TRIM29 与调节 JAK2/STAT3 信号通路的负调节因子 LZTR1 之间存在相互作用关系。进一步研究发现 TRIM29 可促进 LZTR1 的泛素化，进而促进蛋白酶体介导的 LZTR1 的降解，最终激活 STAT3/P-gp 信号轴，从而导致胆囊癌细胞出现化疗耐药性。回复实验表明 STAT3 的抑制剂 HO-3867 能抑制 TRIM29 介导的 STAT3 的激活及 P-gp 的上调，最终增强了胆囊癌细胞对吉西他滨的敏感性。本研究表明 TRIM29 可能是提高胆囊癌吉西他滨化疗敏感性的新靶点，为临床治疗提供新的治疗策略。

生物反应器大规模 3D 扩增人脐带间充质干细胞

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【摘要】人脐带间充质干细胞（Human Umbilical Cord Mesenchymal Stem Cell，hUC-MSCs）因其旁分泌和免疫调节性能良好，可应用于膝骨关节炎、间歇性肺病等疾病的治疗，在炎症相关或自身免疫相关疾病的治疗方面已经进入正式临床试验。目前生产中主要采用培养瓶、培养皿的二维（2D）方式培养 hUC-MSCs，该培养方式需要耗费大量的人力物力，而且较多人为因素介入难以保证其在相关药物制备过程中批次间的稳定性，因此需要一种减少人为干预的大规模培养 hUC-MSC 的方式以满足药物制备生产的需求。针对该问题，本研究以华氏通胶间充质干细胞（Wharton’s Jelly Mesenchymal Stem Cells, WJ-MSCs）为例，应用艾贝泰自主研发的 1L 生物反应器摸索其大规模扩增工艺，培养总体系为 350mL，包含 4×10^7 WJ-MSCs，1g 胶原包被的可溶性微球（Corning）。研究发现，3D 培养开始间歇搅拌能使细胞均匀贴附在微球载体表面，经过 6h 间歇搅拌细胞的贴附率为 75.95%，之后以 55rpm 转速连续搅拌促进细胞生长。细胞培养过程中每日定时采样，通过显微镜观察并结合生物反应器的可视化参数进行监测，依据细胞生长状态执行计数、换液等操作。经计数统计，从细胞贴壁完成后培养至 Day 6，能实现约 10 倍的增殖。此外，本研究还使用 3D 培养的 WJ-MSCs 进行了 C57BL/6 小鼠后背全层皮肤治疗试验，初步试验结果表明该 WJ-MSCs 在皮肤修复中表现较好。

综上所述，本研究摸索出了使用 1L 生物反应器 3D 大规模培养 WJ-MSCs 的培养条件，并对其进行动物试验验证，验证结果表现较好，展现出了工业化培养 WJ-MSCs 与下游应用相结合的潜能。

【关键字】生物反应器；3D 培养；人脐带间充质干细胞；华氏通胶间充质干细胞；

单核细胞促进循环造血干祖细胞扩增和功能

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【摘要】稳态外周血中的循环造血干细胞和祖细胞（cHSPCs）虽然易于获取，但由于它们数量极少并且分子机制不明确，难以用于基础研究和临床应用。在这项工作中，我们开发了一种新的扩增人类 cHSPCs 的单核细胞孵育系统，并成功得到了表型和功能上都处于原始阶段的 CD34+ cHSPCs。该系统使得 $LIN^-CD45RA^-CD34^+CD38^-$ HSPCs 的数量增加了 100 倍，而且扩增的细胞在免疫缺陷小鼠体内能够发挥至少 9 个月的造血功能。通过单细胞测序和无标记定量质谱分析，在转录组和蛋白质组水平上对该系统孵育和非孵育的 cHSPCs 进行了图谱比较。结合因子抑制剂/激动剂测试的综合分析表明，单核细胞孵育对 cHSPCs 扩增的影响可能源于溶酶体酸化和 HIF-1-siganl-dependent 依赖性方式。最后，我们验证了单核细胞孵育也能促进脐带血来源的造血干细胞和祖细胞（UCB-HSPCs）的扩增，并提高 UCB-HSPCs 的重建能力。总之，用于 cHSPCs 扩增的单核细胞孵育系统将促进基于稳态外周血或脐带血疗法的临床应用。

阻断 TSP-1/CD47 信号途径可改善静脉窦/巨核细胞造血龛功能促进供体造血干细胞植入

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【摘要】造血干细胞移植（Hematopoietic stem cell transplantation, HSCT）依然是治疗多种恶性血液病等疾病中应用最广泛、最有效的临床手段。无论放疗或化疗预处理，都会引起严重的 HSCT 相关性内皮损伤综合征。因此，提高 HSCT 疗效并减轻其并发症具有重要的临床意义。

本研究利用放疗预处理的同基因 HSCT 模型，通过阻断 TSP-1/CD47 通路可促进供体植入和造血微环境的改善。野生型小鼠作为受体时，供体细胞在静脉窦/巨核细胞龛（SEC/MK）中鲜有植入。与野生型相比，CD47KO 小鼠中 TSP-1 表达下调，少量血小板黏附在 SEC 上（可能通过 TSP-1/CD36 途径），血小板释放的 CXCL12 进一步招募更多的血小板（$CXCR4^{+}$），表现为静脉窦腔中 CXCL12 的大量聚集以及供体在 SEC/MK 造血龛中的显著聚集。TSP-1KO 小鼠中，由于 TSP-1 的缺失，使得血小板不能够与内皮细胞粘附，血栓形成不稳定，血小板来源的 CXCL12 呈一薄层粘附在 SEC 上，植入的供体分布模式也更为均匀。CD47KO 和 TSP-1KO 受体中，血小板与 SEC 粘附下调，血管损伤减少，HSC 植入后血管再生更优。我们还借助 TSP-1 中和抗体对该机制进行了验证。

TSP-1 作为分泌型蛋白，其功能在人和鼠之间高度保守。因此，推测 TSP-1/CD47 通路可作为提高 HSCT 疗效和改善 HSCT 相关内皮损伤综合征临床转化的有效靶点。

【关键字】TSP-1, CD47, CXCL12，造血干细胞移植，静脉窦内皮细胞/巨核细胞龛

Umbilical cord mesenchymal stem cells promote osteosarcoma cell migration by regulating the p53/MDM2/MAPK signaling pathway through CALB1

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【摘要】Osteosarcoma (OS) is a common primary malignant solid bone tumor. It has a high incidence rate in adolescents and is prone to metastasis. Human umbilical cord mesenchymal stem cells (hUCMSCs), as potential candidate stem cells for cell and gene therapy, have homing ability and affect the occurrence and metastasis of various tumors, but their impact on OS is not clear. In this study we have confirmed that hUCMSCs conditioned medium (CM) can promote the migration of OS cells through wound healing assay and transwell migration assay. RNA-Seq sequencing analysis showed that CALB1 gene was significantly up-regulated in OS cells treated with hUCMSCs-CM. KEGG analysis enriched p53-MDM2 and MAPK signaling pathways related to CALB1. Based on the above research results, we believe that there is a correlation between CALB1 gene expression and OS cells migration, and hUCMSCs may promote OS cells migration by regulating the CALB1 gene and its downstream p53/MDM2/MAPK signaling pathway. This study will provide new target references and research foundation for hUCMSCs therapy of OS.

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【关键字】osteosarcoma; human umbilical cord mesenchymal stem cells; CALB1; migration

小分子化合物激活 Wnt 信号通路促进人脐带间充质干细胞软骨分化

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【摘要】目的：利用小分子化合物持续激活或抑制 Wnt 信号通路来评估其对人脐带间充质干细胞软骨分化的作用效果。

方法：利用能够特异性激活和抑制 Wnt 信号通路的两种小分子化合物 CHIR99021 和 XAV939，在人脐带来源的间充质干细胞（hUCMSCs）软骨诱导过程中调控 Wnt 信号通路活性。首先设置不同浓度梯度的 CHIR99021 和 XAV939 培养 hUCMSCs，48 h 后收集细胞并通过 qRT-PCR 检测 Wnt 下游基因 LEF1 的表达，以确定 CHIR99021 和 XAV939 的有效作用浓度。随后将确定浓度的小分子化合物添加到软骨诱导培养基中，在 hUCMSCs 软骨诱导过程中持续激活或抑制 Wnt 信号通路。通过 qRT-PCR 检测各组细胞软骨标志基因 SOX9 以及肥大标志基因 COL10A1 和 ALP 的表达。软骨诱导 21 d 后使用阿尔新蓝-核固红对细胞进行染色，比较 CHIR99021 和 XAV939 对软骨细胞中的酸性粘多糖蛋白表达情况影响。

结果：本研究首先建立了 hUCMSCs 并完成鉴定。浓度梯度实验确定了 3 μM CHIR99021 和 30 μM XAV939 分别能够有效激活和抑制 hUCMSCs 中的 Wnt 信号通路活性。与常规软骨诱导组相比，虽然两种小分子化合物的添加均对软骨标志基因 SOX9 和肥大标志基因 COL10A1 表达无影响，但在软骨诱导过程中使用 CHIR99021 激活 WNT 信号通路能够降低肥大标志基因 ALP 的表达。阿尔新蓝染色结果显示，与对照组相比，使用 CHIR99021 激活 Wnt 信号通路后软骨细胞中酸性粘多糖蛋白明显增多，而通过 XAV939 抑制 Wnt 信号通路后则减少了酸性粘多糖蛋白表达。以上结果证实了使用适当浓度的小分子化合物激活 Wnt 信号通路能促进 hUCMSCs 软骨分化。

结论：小分子化合物 CHIR99021 在 3 μM 浓度下可有效激活 hUCMSCs 中 Wnt 信号通路并且能够降低肥大基因 ALP 表达，增加酸性粘多糖蛋白表达，有利于 hUCMSCs 软骨分化。

【关键字】人脐带间充质干细胞；软骨分化；小分子化合物；WNT 通路

安罗替尼加入早期三阴性乳腺癌术前新辅助化疗的有效性和安全性

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【摘要】From Jan 2021 to Aug 2022, 48 patients were assessed and 45 were enrolled. All patients received at least one dose of study treatment and underwent surgery. The median age was 48.5 years (SD: 8.7), 71% were nodal involved, and 20% had stage III. In the intention-to-treat population, 26 out of 45 patients achieved pCR (57.8%; 90% CI, 44.5%–70.3%), and 39 achieved residual cancer burden class 0-I (86.7%; 95% CI, 73.2%–94.9%). The bpCR and apCR rate were 64.4% (29/45) and 71.9% (23/32), respectively. No recurrence or metastasis occurred during the short-term follow-up. Based on the FUSCC IHC-based subtypes, the pCR rates were 68.8% (11/16) for immunomodulatory subtype, 58.3% (7/12) for basal-like immune-suppressed subtype and 33.3% (4/12) for luminal androgen receptor subtype, respectively. NGS revealed that the pCR were 77% (10/13) and 50% (14/28) in MYC-amplified and wild-type patients, respectively, and 78% (7/9) and 53% (17/32) in gBRCA1/2-mutated and wild-type patients, respectively. The median follow-up time of the study was 14.9 months (95% CI: 13.5–16.3 months). There was no disease progression or death during neoadjuvant therapy. No deaths occurred during postoperative follow-up. In the safety population (N = 45), Grade 3 or 4 treatment emergent adverse events occurred in 29 patients (64%), and the most common events were neutropenia (38%), leukopenia (27%), thrombocytopenia (25%), anemia (13%), and hypertension (13%), respectively.

shRNA 靶向结合慢病毒载体负链 mRNA 进而提高病毒包装滴度的研究

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【摘要】β-地中海贫血是一种隐性单基因遗传病，主要是由于β珠蛋白基因合成缺陷导致α-和β-珠蛋白链比例失衡所致，基因与细胞治疗是根治该疾病的最佳解决方案。慢病毒载体（LVV）已成为临床试验中基因治疗的常用载体之一。据报道，LVV 介导的临床试验已经成功地治疗了近百例β-地中海贫血病例。这些 LVVs 携带一个反向放置的β-珠蛋白（HBB）基因表达盒，以在病毒 RNA 包装过程中使其携带的内含子得以保存。因此，这些 LVV 往往在其包装过程中产生由其红系特异的启动子驱动的少量负向转录本，并通过与病毒主链互补而降低病毒滴度。为了克服这一问题，我们设计了专门针对由 LVV 内部启动子驱动的负链转录本的 shRNA，从而显著增加了病毒包装滴度。这一研究显示了一个简单且有效的方法来提高慢病毒载体的包装滴度，进而提升β-地中海贫血基因治疗的效果。

【关键字】慢病毒载体，滴度，RNA 干扰，BB305，反向表达盒

Human Induced Pluripotent Stem Cells derived Neutrophils Display Strong Anti-microbial Potencies

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【摘要】Neutrophils are essential innate immune cells with unusual anti-microbial properties while dysfunctions of neutrophils lead to severe health problems such as lethal infections. Generation of neutrophils from human induced pluripotent stem cells (hiPSCs) is highly promising to produce off-the-shelf neutrophils for transfusion therapies. However, the anti-microbial potencies of hiPSCs derived neutrophils (iNEUs) remain less documented. Here, we develop a scalable approach to generate iNEUs in a chemical defined condition. iNEUs display typical neutrophil characters in terms of phagocytosis, migration, formation of neutrophil extracellular traps (NET) etc.. Importantly, iNEUs display a strong killing potency against various bacteria such as K. pneumoniae, P.aeruginosa, E.coli, S.aureus etc.. Moreover, transfusions of iNEUs in mice with neutrophil dysfunction largely enhance their survival in lethal infection of different bacteria. Together, our data show that hiPSCs derived neutrophils hold strong anti-microbial potencies to protect severe infections under neutrophil dysfunction conditions.

【关键字】干细胞；中性粒细胞；细胞治疗

七种 CD19 CAR 设计在工程化 NK 细胞中的抗肿瘤活性比较

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【摘要】CAR-NK 细胞疗法的安全性和通用性具有显著优势，在癌症治疗领域展现出了巨大的应用前景。目前，制备 CAR-NK 细胞的 CAR 结构大都只在 CAR-T 细胞中得到了功能评估。考虑到 NK 细胞与 T 细胞的差异，因此仍需要优化专门针对 NK 细胞的 CAR 结构，以提高其治疗效果。本研究中，我们比较了利用 CD19 scFv 制备的七种 CD19 CAR 结构，其中 CAR 1（CD8 TMD-CD3ζ SD），CAR2（CD8 TMD-FcεR I γ SD），CAR3（CD8 TMD-OX40 CD-CD3ζ SD），CAR5（CD28 TMD-FcεR I γ SD）和 CAR6（CD8 TMD-4-1BB CD-CD3ζ-1 ITAM SD）结构源于文献报道，CAR 4（CD8 TMD-OX40 CD-FcεR I γ SD）和 CAR7（CD8 TMD-OX40 CD-CD3ζ-1 ITAM SD）是本研究中改造设计的。利用逆转录病毒系统将这七种 CD19 CAR 结构在脐带血来源的 NK 细胞中进行过表达，进而全面评估这七组 NK 细胞的扩增能力、靶向肿瘤杀伤活性和体内持久性。首先，只有 CAR4 削弱了 NK 细胞的扩增能力，其它 6 种 CD19 CAR 结构对 NK 细胞的扩增没有影响；在体外靶向杀伤评估中，CAR1-NK 细胞表现出最有效的靶向杀伤肿瘤的活性，尤其是在较低的效靶比情况下，CAR1-NK 细胞表现更为突出。而 CAR5-NK 细胞杀伤效率最低。在与靶细胞长期接触后，CAR7-NK 细胞表达耗竭相关标记物的表达增加最高。在体内抗肿瘤评估中，仍然只有 CAR1-NK 细胞在治疗异种移植肿瘤小鼠方面表现出优越的疗效。然而在 CAR1 的基础上组合 OX40 共刺激结构域形成的 CAR3 能够使 NK 细胞（即 CAR3-NK 细胞）在体内表现出更显著的持久性。而 CAR5-NK 细胞的体内抗肿瘤效果最差。本研究为开发用于临床治疗的 CAR-NK 细胞产品提供了重要的参考。

hESC 来源通用型 CD19 CAR-iNK 细胞抗 B 细胞肿瘤研究

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【摘要】CAR-NK 细胞输注无需供者 HLA 配型、毒副作用小，有望成为现货式免疫治疗细胞产品。然而，异基因 CAR-NK 细胞在输注后很容易被患者的免疫系统清除，导致 CAR-NK 细胞输注后在体内的持续性短、缺乏长期疗效。本研究提供了一种高效稳定制备通用型 CD19 CAR-iNK 细胞的策略，这种通用型 CD19 CAR-iNK 细胞不仅可以避免异体 T 细胞和 NK 细胞的排斥，还能高效杀伤 B 肿瘤细胞。首先，我们构建了 B2M（HLA-I 类分子的亚基）敲除、HLA-E 和 CD19 CAR 过表达的人胚干细胞系（CD19 CAR-UESC）。结合类器官培养方法将其诱导分化为通用型 CD19 CAR-NK 细胞（CD19 CAR-UiNK）。单细胞测序及流式分析结果显示，CD19 CAR-UiNK 细胞高表达 NK 细胞的激活和抑制性受体，以及 NK 细胞相关的关键效应分子。由于缺失 HLA-I 类分子，CD19 CAR-UiNK 细胞不会引起异体 CD8+ T 细胞介导免疫排斥反应。同时，HLA-E 的表达能够一定程度上抑制 HLA-I 分子下调导致的 NK 细胞攻击。在与 Nalm-6 肿瘤细胞共培养的情况下，CD19 CAR-UiNK 细胞的 IFN-γ和 TNF-α分泌水平相对于 iNK 细胞显著增加，且 CD107a 表达也显著升高。功能上，CD19 CAR-UiNK 细胞能够在体外有效清除 CD19+肿瘤细胞，特别是来自患者的原代 B 肿瘤细胞。同时，CD19 CAR-UiNK 细胞在 Nalm-6 肿瘤动物模型中表现出较强的抗肿瘤活性，能够有效抑制 Nalm-6 肿瘤细胞进展，并显著延长肿瘤负荷小鼠生存期。本研究对开发通用型、现货式 CAR-NK 细胞产品治疗肿瘤患者提供了重要参考。

【关键字】B2M HLA-E CD19 CAR-iNK

Gremlin1-MSCs 改善 PDA 涂层小口径聚氨酯人工血管通畅性的研究

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【摘要】目的：小口径人工血管（< 6 mm）由于早期血栓形成和远期内膜增生，导致远期通畅率低，制约其临床应用。本研究构建过表达 Gremlin1 的间充质干细胞（Gremlin1-MSCs）并种植人工血管内膜，旨在探究其作为种子细胞对小口径人工血管血栓形成和内膜增生的影响，为改善小口径人工血管通畅性提供新的实验依据。

方法：慢病毒转染构建 Gremlin1-MSCs，鉴定其 MSCs 性质并检测 Gremlin1 表达。Transwell 构建人髓性白血病单核细胞（THP-1）和 Gremlin1-MSCs 共培养体系，检测 THP-1 的增殖和极化情况。聚多巴胺（PDA）涂覆小口径聚氨酯（PU）人工血管内表面进行改性，并检测该涂层对细胞种植的影响。家兔行颈总动脉-人工血管置换术，分别置换种植 Gremlin1-MSCs，Ctrl-MSCs 和无细胞种植的人工血管，明确 Gremlin1-MSCs 种植对人工血管通畅性的影响。

结果：流式细胞术和三系分化（成脂、成骨、成软骨）结果显示 Gremlin1-MSCs 保持间充质干细胞的表型和功能；免疫荧光染色和荧光定量 PCR 证实了 Gremlin1 的过表达。免疫荧光染色、CCK8 和荧光定量 PCR 结果表明 Gremlin1-MSCs 可抑制 THP-1 增殖和从 M0 型向 M1 型巨噬细胞极化，抑制促炎因子 TNF-α 和 IL-6 的表达，促进抑炎因子 IL-10 和 CCL22 的表达。接触角检测显示 PDA 涂层增强了人工血管的亲水性；免疫荧光染色结果表明 PDA 涂层增强了细胞在人工血管表面的粘附和增殖能力；细胞毒性和溶血性实验结果表明涂覆后的人工血管具有良好的生物相容性和血液相容性。家兔颈总动脉-人工血管置换后，彩色多普勒超声显示 Gremlin1-MSCs 组人工血管血流速度最快；扫描电镜显示 Gremlin1-MSCs 组人工血管内壁细胞覆盖比率显著高于 Ctrl-MSCs 组和无细胞种植组。HE 和 Masson 染色显示 Gremlin1-MSCs 种植组人工血管管腔横切面中血栓占比最少，表明 Gremlin1-MSCs 种植能更好地抑制血管内膜增生。

结论：Gremlin1-MSCs 种植可显著抑制血栓形成及内膜增生，改善小口径人工血管的通畅性，PDA 涂层可增强细胞在人工血管上的粘附，为 Gremlin1-MSCs 发挥作用提供保障。本研究为小口径人工血管的临床应用提供了新的思路和实验依据，具有广阔的应用前景。

【关键字】小口径人工血管，Gremlin1，间充质干细胞，内膜增生，聚多巴胺

四、干细胞相关前沿进展

AMPA 受体突触可塑性与相关脑疾病

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【摘要】AMPA 受体（AMPARs）介导中枢神经系统中大部分快速兴奋性突触传递。AMPA 受体插入和离开突触的动态过程在突触可塑性以及学习记忆中发挥至关重要的作用。我们实验室的长期目标是阐明健康和疾病状态下大脑中突触可塑性和学习记忆的神经基础。利用慢性不可预测性温和应激（CUMS）诱导小鼠抑郁模型，我们发现突触体中的 AMPA 受体水平和基底核杏仁核（pBLA）到腹内侧前皮质（vCA1）的连接在 CUMS 诱导的抑郁样行为中起着关键作用，并发现大麻二酚（CBD）有望成为抑郁症治疗的新靶点。这一发现为从 AMPA 受体突触可塑性和在神经环路水平更好地理解抑郁障碍的病理生理学提供了新的视角。利用 SEP-GluA1 敲入鼠与 5xFAD 小鼠杂交构建了荧光标记内源 AMPA 受体 GluA1 亚基的 AD 模型鼠，我们发现 5xFAD 小鼠的学习缺陷与初级感觉皮层突触体整体 GluA1 蛋白水平增加的缺失相关。双光子活体成像结果进揭示特定亚群神经元树突棘表面 GluA1 及树突棘大小增加的缺失与学习缺陷直接相关。这些异常可能由 5xFAD 小鼠中小胶质细胞和星形胶质细胞过度活化引起。我们的研究实时观察了 AD 小鼠学习过程中的内源性 AMPA 受体动态，为理解 AD 小鼠学习障碍的突触机制提供了实验基础，并为更好地理解 AD 发病机制提供了线索。

TX1 调节胎儿血红蛋白

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Abstract

Fetal globin genes, which are turned off after birth, can be reactivated in adults to improve symptoms of sickle cell disease and β-thalassemia. Here, via a CRISPR-Cas9–guided loss-of-function screen in human erythroblasts, we identify transcription factor TX1, known to be involved in transcription, as a novel g-globin regulator.Specificity In adult human erythroid cell cultures, loss of TX1 leads to the reactivation of fetal hemoglobin genes. This disruption could provide new avenues for treating hemoglobin disorders.

Result

To identify novel regulators of HbF expression, we conducted a genome-wide sgRNA library screening. The sgRNA library contains sgRNAs targeting 12,0000 genes, with 6 sgRNAs designed for each gene. After staining for HbF, we sorted the top and bottom 10% of HbF-expressing cells and evaluated the representation of each sgRNA through deep sequencing. As anticipated, sgRNAs targeting the known γ-globin repressors ZBTB7A and HRI were significantly enriched in the HbF-high population, confirming the effectiveness of our screening process. Notably, all 6 sgRNAs in the library targeting TX1, were enriched in the HbF-high cells suggesting that TX1 may function as a direct or indirect repressor of γ-globin expression. (Figure 1)

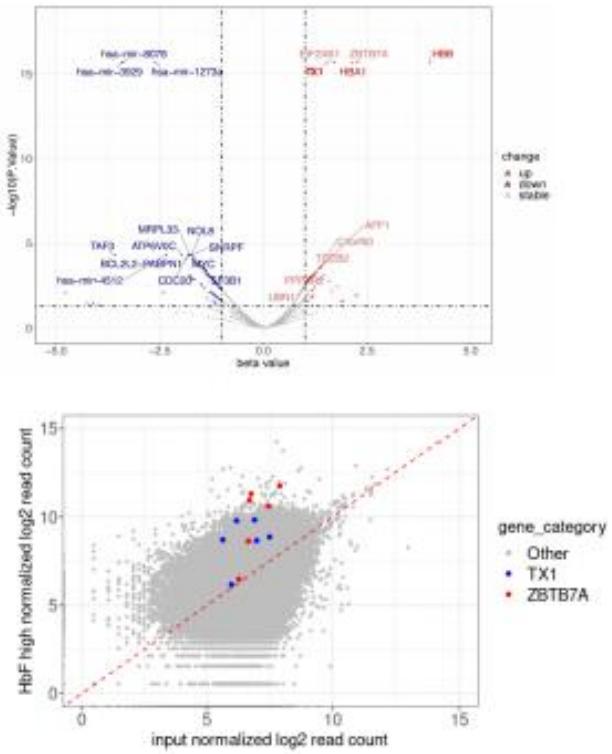

Figure 1. CRISPR screen identifies TX1 as a novel g-globin regulator

To validate the screening results, two independent sgRNAs targeting TX1 were stably introduced into HUDEP-2-Cas9 cells along with a positive control sgRNA (targeting ZBTB7A) and non targeting negative control sgRNA. Depletion of TX1strongly increased the fractionof HbF-expressing cells, as determined by flow cytometry using anti-HbF antibodies (Figures 2).

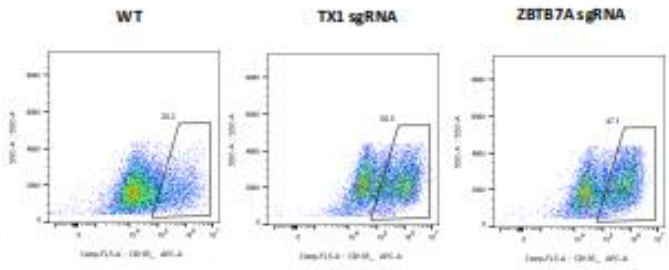

Figure 2. TX1 depletion elevates γ-globin in HUDEP2 cells.

Conclusion:

Taken together, these results suggest TX1 as a potential therapeutic target for hemoglobinopathies.

Trace amine-associated receptor 1 regulates neurocircuitry via NMDAR in a glutamatergic cortical organoid

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【摘要】Trace amine-associated receptor 1 (TAAR1), an important G protein-coupled receptor expressed in the mammalian brain, recognizes a range of biogenic amines to modulate dopaminergic and glutamatergic neurotransmission in adult brains. Recently, the selective activation of TAAR1 has shown promising therapeutic potentials for drug addiction and neurological disorders in adult animal models. While its poly-pharmacological actions made it challenging to understand the role of TAAR1 in human brain development. Here, we developed a glutamatergic neuron-enriched cortical organoid model (gCO) using human pluripotent stem cells and explored the function of TAAR1 in neurogenesis in vitro. Briefly, mature gCOs (differentiation day 60-150) were identified with enriched expression of glutamatergic neuronal markers, vGLUT1, MAP2, TUJ1, and SYP, as well as neurotrophic glial cell marker S100ß. Glutamate-responsive calcium traces and electrophysiological spikes further confirmed the mature glutamatergic neuron function in gCOs. Furthermore, a low level of TAAR1 was detected in mature gCOs. Compared with the vehicle control group, short-term TAAR1 activation potentiated the mutual connection between neighbor neurons in the high frequency detection pattern of calcium imaging, and vice versa. Long-term activation and inhibition TAAR1 brought the complete inhibition of neural connection across the whole spheroid and TAAR1 inhibition (more than 60 days) even lead to a cracked appearance, compared to the gradually refined electrophysiological performances and intact appearance of control organoids. The hypofunction of N-methyl-D-aspartate receptor (NMDAR), closely correlated with glutamate function, in two long-term TAAR1-dysregulated groups might be the leading cause for the neural deficits supported by transcriptomic sequencing analysis. Our study indicates that TAAR1 plays a crucial role in the glutamate neural circuitry during cortical development. These findings remind that the TAAR1-targeted therapeutics should be developed under the consideration of the negative influences on neural function in the paradigm of brain development.

【关键字】Trace amine-associated receptor 1; glutamatergic neuron; cortical organoids; calcium

activity; electrophysiology

基于维持端粒稳定防止人脐带间充质干细胞衰老的新策略

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【摘要】人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUC-MSCs)治疗是通过免疫、炎症的调节以及细胞外囊泡(EVs)等方式，最终实现延缓机体的衰老。然而由于细胞多次复制、氧化应激等因素，导致进入衰老状态，其特征是细胞功能下降，生长速率变慢等。由于 hUC-MSCs 在体外培养后容易衰老，因此如何获取大量健康的 hUC-MSCs 仍然是干细胞应用到医学领域的挑战之一。本研究采集刚分娩的孕妇脐带，使用组织块分离法取得人脐带间充质干细胞。之后在低氧气浓度(5% O2)的培养条件下，并联合维生素 C (VC)和 N -乙酰半胱氨酸(NAC)处理 hUC-MSCs，培养至第十代（P10）后通过细胞增殖、端粒荧光原位杂交（Q-FISH）、β-半乳糖苷酶染色和细胞转录组测序（RNA-seq）等方法检测细胞衰老的相关指标。实验结果显示对照组在 P7 代细胞出现衰老状态，而在低氧条件下 VC 和 NAC 处理的 P10 代细胞增殖速度和 P3 代细胞保持一致，并维持了端粒长度。RNA-seq 结果也显示 VC 和 NAC 处理后可显著抑制细胞衰老相关指征。GO 和 KEGG 通路主要富集在细胞衰老、核糖体生物合成、DNA 复制、染色体区域和细胞周期等通路。如衰老标志基因 CDKN1A（P21）、CDKN2A（P16）、IL-1α、IL-6、TP53 和 TGFβ1 等在处理组表达下降，MRE11、ZFP36L1、FOXM1 和 RB1 等在处理组表达上升。总之 VC 和 NAC 以及低氧处理可实现 hUC-MSCs 在体外长期稳定的培养，为 hUC-MSCs 的临床应用提供重要基础和理论依据。

【关键字】人脐带间充质干细胞，端粒，衰老

复合乙酰化葡甘聚糖电纺膜在干细胞定向分化中的应用

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【摘要】Stem cells provide great potential for replenishment of the neuronal population lost. However, the difficulty in obtaining sufficient number and poor survival of terminal motor neurons limit its application in stem cell therapy. To address these issues, we have previously constructed a pluripotent stem cell line has normal proliferation ability, and can differentiate into motor neurons both in vitro and in vivo. However, poor survival is in non-immunodeficient motor neuron disease models, making it difficult to exert therapeutic effects.

Acetylated konjac glucomannan (acGM) has anti-inflammatory property. The acGM electrospun membrane provides adhesion and scaffold functions. The sustained-release function of the acGM electrospun membrane will also facilitate drug delivery to the central nervous system, bypassing the blood-brain barrier.

Therefore, we aim to create a multi-functional composite material with anti-inflammatory, adhesive scaffold, and drug sustained-release properties. We will seed genetically modified stem cells onto the electrospun membrane. Under the action of doxycycline, these cells will be directed to differentiate into motor neurons, and under the action of cyclosporine, immune rejection will be inhibited.

【关键字】干细胞；复合乙酰化葡甘聚糖；脑创伤；神经分化

Therapeutic In Vivo Gene Editing Achieved by a Hypercompact CRISPR-Cas12f1 System Delivered with All-in-One Adeno-Associated Virus

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【摘要】CRISPR-based gene therapies are making remarkable strides toward the clinic. But the large size of most widely-used Cas endonucleases including Cas9 and Cas12a restricts their efficient delivery by the adeno-associated virus (AAV) for in vivo gene editing. Being exceptionally small, the recently engineered type V-F CRISPR-Cas12f1 systems can overcome the cargo packaging bottleneck and present as strong candidates for therapeutic applications. In this study, the pairwise editing efficiencies of different engineered Cas12f1/sgRNA scaffold combinations are systemically screened and optimized, and the CasMINI_v3.1/ge4.1 system is identified being able to significantly boost the gene editing activity. Moreover, packaged into single AAV vectors and delivered via subretinal injection, CasMINI_v3.1/ge4.1 achieves remarkably high in vivo editing efficiencies, over 70% in transduced retinal cells. Further, the efficacy of this Cas12f1 system-based gene therapy to treat retinitis pigmentosa in RhoP23H mice is demonstrated by therapeutic benefits achieved including rescued visual function and structural preservation. And minimal bystander editing activity is detected. This work advances and expands the therapeutic potential of the miniature Cas12f1 system to support efficient and accurate in vivo gene therapy.

【关键字】CRISPR-Cas12f1, AAV delivery, gene editing, gene therapy, retinitis pigmentosa

IGF1R signaling influences the generation of reparative macrophages

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【摘要】Macrophages are essential for restoring tissue homeostasis and promoting tissue regeneration following infection and tissue injury. However, the molecular mechanisms regulating reparative macrophage formation remain unclear. Utilization of an acute liver injury model, we found that reparative macrophages rely on oxidative phosphorylation (OXPHOS). Blockade of the mitochondrial respiratory chain in macrophages significantly inhibited the generation of reparative macrophages and retarded liver repair. Macrophages acquired OXPHOS by phagocytizing dead cells at the site of liver injury. Such a process can be regulated by insulin-like growth factor-1 receptor (IGF1R) signaling. At the stage of tissue repair post liver injury, an enhancement of insulin like growth factor1 (IGF1) in liver can be observed that enhanced the phagocytosis of macrophages through the IGF1R-PI3K/AKT signaling pathway. Knockdown of IGF1R in macrophages suppressed phagocytosis, impeded the metabolic reprogramming of OXPHOS, and suppressed the liver repair. Taken together, targeting IGF1R can metabolically reprogram reparative macrophages and promote tissue repair.

【关键字】macrophages, IGF1, phagocytosis, mitochondrial metabolism

Anti-PD-1 抗体治疗后肿瘤浸润 T 细胞的差异蛋白质组研究

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【摘要】研究背景：恶性肿瘤严重威胁人类健康。免疫检查点抗体已展现出良好的治疗效果，部分患者能够响应 anti-PD-1 或 anti-PD-L1 的治疗，肿瘤得到有效控制。研究表明，肿瘤浸润 T 细胞响应免疫检查点抗体治疗时，可能同时存在多种机制，以逆转 T 细胞的耗竭状态并促进 T 细胞的杀伤能力。但是，目前仍有部分患者无法有效响应免疫检查点抗体疗法。进一步解析肿瘤浸润 T 细胞响应免疫检查点抗体治疗的分子机制迫在眉睫。

研究目的：检测响应 anti-PD1 抗体治疗的肿瘤浸润 T 细胞中蛋白质组的变化，探索 T 细胞响应抗体免疫治疗的分子机制。

研究方法：本研究构建了基于小鼠淋巴瘤细胞系 E.G7-OVA 的皮下移植瘤动物模型。在 B6 小鼠皮下接种 1 x 10^5 个 E.G7-OVA 细胞，8 天后尾静脉注射 1 x 10^6 CD8+ CD62L+ CD44- 的 OT1 细胞，同时将小鼠分两组，一组小鼠每只腹腔注射 75 ug anti-PD1 抗体，另一组小鼠每只腹腔注射等量的对照 IgG，每 3 天注射一次，连续注射 5 次。最后，分选肿瘤浸润 T 细胞，流式染色分析细胞表面耗竭相关蛋白，同时对部分细胞进行微量蛋白质组质谱分析鉴定，比较 anti-PD1 抗体治疗后的差异蛋白质组。

研究结果：和对照组相比，anti-PD-1 抗体处理组的小鼠的肿瘤体积更小，肿瘤中抗原特异性的 OT1 T 细胞在 CD8+ T 细胞中的占比更高，数目更多，同时细胞表面的耗竭相关蛋白 PD-1 的比例和 MFI 也更低。微量蛋白质组的质谱鉴定结果表明，和对照组相比 anti-PD-1 抗体处理组的小鼠肿瘤中抗原特异性的 CD8+ OT1 T 细胞中，上调表达的蛋白有 392 个，下调表达的有 242 个。其中，在 anti-PD-1 抗体处理组中上调表达的蛋白有杀伤肿瘤细胞的效应因子 Granzyme A 和 Granzyme B，促进 T 细胞效应功能的转录因子 TBX21，以及指示细胞增殖状态的蛋白 MKI67。而下调表达的蛋白中有介导细胞凋亡的蛋白 Caspase-3 以及负调控 TCR 信号转导通路的 E3 泛素连接酶 CBLB。研究结论：本研究成功构建了 anti-PD-1 抗体治疗的肿瘤模型，anti-PD-1 抗体治疗可以有效抑制肿瘤细胞生长，促进肿瘤浸润 T 细胞的增殖，抑制其凋亡，增强其杀伤能力，同时改善肿瘤浸润 T 细胞的耗竭状态。基于差异蛋白质组的进一步关联分析有望发现新的免疫治疗靶点。

【关键字】Anti-PD-1 抗体治疗；肿瘤浸润 T 细胞；蛋白质组

选择性多聚腺苷酸化调控因子 NUDT21 在维持 Naive T 细胞稳态中的功能研究

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【摘要】研究背景：维持 Naive T 细胞稳态，对适应性免疫系统发挥正常功能至关重要。选择性多聚腺苷酸化（Alternative polyadenylation, APA）作为一种转录后修饰的调控机制，其是否参与 Naive T 细胞的稳态维持尚不清楚。RNA 结合蛋白 NUDT21 是 APA 的核心调控元件，能够识别并结合 pre-mRNA 上 3’ UTR 区域的 UGUA 基序，进而招募剪接加尾复合物，并促使 mRNA 在远端位点加尾，产生更长的 3’ UTR。以 NUDT21 为切入点，探究 APA 在维持 Naive T 细胞稳态中的功能，对发现新的免疫治疗靶点将具有重要意义。

研究目的：明确 APA 是否参与 Naive T 细胞的稳态维持，探究 NUDT21 在维持 Naive T 细胞稳态中的功能。

研究方法：构建 Nudt21fl/fl×CD4cre 条件性敲除小鼠，检测 IL-7/IL-7R 信号通路蛋白表达，IL-7 体外培养比较 Naive T 细胞的存活能力，Rag1-/-小鼠体内培养比较 CD4 Naive T 细胞的稳态增殖能力。

研究结果：Nudt21 条件性敲除小鼠的胸腺 T 细胞发育未受影响；脾脏和淋巴结中 CD4 和 CD8 T 细胞的比例和数目均降低；CD4 和 CD8 T 细胞中 Naive 比例降低、Effector 比例升高；CD4 和 CD8 Naive T 细胞的 CD127 和 Bcl2 的 MFI 均降低；IL-7 体外培养 CD4 和 CD8 Naive T 细胞 24h 后 Annexin V 阳性的比例更高；CTV 标记的 WT 和 cKO CD4 Naive T 细胞按 1:1 尾静脉注射 Rag1-/-小鼠 7 天后，cKO CD4 Naive T 细胞的比例降低，CTV low 的比例也更低。研究结论：本研究发现 APA 参与 Naive T 细胞的稳态维持，NUDT21 在维持 Naive T 细胞稳态平衡中具有重要作用。NUDT21 缺失导致 Naive T 细胞稳态失衡，IL-7/IL-7R 信号通路受损，外周 Naive T 细数目减少，稳态增殖能力下降。基于本研究的结果，靶向转录后修饰机制将有助于发现新的免疫治疗靶点，并为免疫缺陷疾病、免疫衰老、抗肿瘤免疫以及疫苗免疫反应等研究提供新的视角。

【关键字】Naive T 细胞稳态；选择性多聚腺苷酸化；NUDT21

Adaptation Dynamics and Maternal-Fetal Interactions Recapitulating Human Embryo Implantation via a 3D Co-culture Modeling

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【摘要】Human embryo implantation is a highly dynamic and transient process orchestrated by sophisticated interplays between embryo and endometrium. Studying this crucial phase in-vitro has been limited by technical and ethical challenges. Here, we develop a 3D co-culture model by implanting blastoids into endometrial assembloids, which effectively simulates human in-vivo implantation and enables detailed exploration of maternal-fetal dynamics. Our system unveils implantation-triggered subpopulations, such as pioneering trophoblasts from blastoid derivatives that acquire endometrial characteristics, and highly specialized ciliated epithelia within the endometrium that shed epithelial properties. The emergence of these subpopulations demonstrates active adaptations, responding coordinately to successful embryo implantation from both fetal and maternal perspectives. Additionally, our system highlights the critical roles of diverse heterologous cell-cell contacts, extracellular matrix interactions and secreted signaling in mediating embryo adhesion and invasion. Overall, our study offers fresh insights into the mechanisms of embryo development and provides potential targets for reproductive research.

A single morphogen signaling center-guided human gastrula model

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【摘要】The crucial signaling hub at the posterior end of the gastrula plays a pivotal role in shaping vertebrate body patterns, yet its involvement in human embryogenesis remains uncharted due to ethical restrictions. Here, we successfully derived an artificial morphogen signaling center from human embryonic stem cells by combined WNT and Nodal signaling. Upon assembly with aggregates of human embryonic stem cells, this signaling hub functions as an organizer, orchestrating the anterior-posterior symmetry breaking and elongation of the system. Furthermore, it governs posterior development, including multilineage differentiation such as endoderm, mesoderm, ectoderm, neural and neuromesodermal progenitors, as well as specialization of posterior tissue and organ, such as a hindgut-like structure organized arrangement of endodermal cells, and an allantois-like luminal structure specification. Single-cell transcriptomic analyses show that this system faithfully recapitulates the human gastrulating embryo at the Carnegie stage 7-8, highlighting the crucial role of the posterior signaling center in guiding early gastrulation. Additionally, by disrupting FOXH1 in signaling hub, we replicate the embryonic malformation associated with Foxh1 deficiency in mice, including aberrant gross morphology and disordered endoderm cells sorting. In summary, this modular and manipulable system provides valuable insights into the complex and coordinated processes orchestrated by the posterior signaling hub and holds promise for investigating the regulatory roles of other signaling centers in embryonic development.

【关键字】Organizer, Human gastrula model, Embryonic stem cells, FOXH1

iPSC 来源抗 EGFRvIII 嵌合抗原受体巨噬细胞在胶质瘤治疗中的应用

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【摘要】 Mφ高度浸润是实体肿瘤免疫微环境的重要特征；CAR-M 细胞治疗是肿瘤免疫治疗新思路。利用 Mo/Mφ向肿瘤归巢的特质，研发 iPSC 来源 CAR-M 的高效制备方法并构建逆转肿瘤免疫抑制微环境的 CAR-M 免疫治疗模型，有望成为实体肿瘤免疫治疗新策略。外周 Mφ扩增能力弱和基因编辑效率低是限制 CAR-M 应用的技术瓶颈。本研究利用 iPSC 这一扩增高效、编辑易行且具备多向分化潜能的干细胞作为种子细胞，研发高效诱导 iPSC 定向 Mφ分化的关键技术，构建 CAR-M 高效制备体系，探究将其用于 GBM 免疫治疗的新策略。

本研究构建 B2M 敲除且 HLA-E 定点敲入的“通用型” iPSC 株，不影响正常造血分化；研发了玻连蛋白介导的单层诱导早期造血分化体系，构建了 3 株稳定表达 CD19-CAR 的 CAR-iPSC 细胞株，提高了 iPSC 来源 CAR-M 制备效率（1 个 iPSC 平均产生 3000-6000 个 Mφ），提升 Mφ分化效率至 90%以上，缩短制备周期至 21 天；通过标记物检测及动态的转录组表达对 CAR-M 进行表型鉴定和体系质控；构建 CAR-M 和肿瘤细胞体外共培养模型和 CAR-M 回输免疫缺陷小鼠模型，表明 iPSC-CAR-M 能在体外和体内有效杀伤 B 淋巴瘤和实体肿瘤细胞。

同时，筛选鉴定出靶向 GBM 的肿瘤特异性抗原靶点 EGFRvIII，构建第二代 CAR-M 结构，制备了靶向 EGFRvIII 的 CAR 载体。构建了在胞内信号转导结构域后串联 FcγRI 链或 FcγRIIA/C 链，优化逆转免疫抑制的 CAR 模型。在 THP-1 细胞模型中验证第二代 CAR-M 有效增强了吞噬作用，可诱导 CAR-M 炎性活化。

【关键字】iPSC；CAR-M；EGFRvIII；胶质瘤

Therapeutic Potential of Umbilical Cord Mesenchymal Stem Cells (UCMSCs) and Super Activated Platelet Lysate (sPL) in Enhancing Endometrial Regeneration in Rats with Thin Endometrium

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【摘要】Current investigations underscore the widespread utilization of MSCs in preclinical and clinical settings, encompassing distinct types such as bone marrow mesenchymal stem cells, adipose mesenchymal stem cells, and umbilical cord blood mesenchymal stem cells. UCMSCs have demonstrated efficacy in promoting tissue regeneration and repairing damaged endometrium in various preclinical applications to address thin endometrium-related therapeutic challenges. sPL can enhance hormone secretion, directly activate MSCs and expedite tissue regeneration. Consequently, stem cell and cytokines combination therapy as promising modalities for addressing thin endometrium and, thereby, augmenting the likelihood of successful pregnancies. We performed comprehensive histological, biological, and functional analyses to explore the potential of sPL or UCMSCs administration in reinstating endometrial functionality and enhancing pregnancy outcomes. This study aimed to establish a rat model of thin endometrium and investigate the effects of super-activated platelet lysate (sPL) and umbilical cord mesenchymal stem cells (UCMSCs) on the thin endometrium in rats. sPL is collected from the platelet through ultra-low temperature freeze-thawing and activated by adding 10% CaCl2. UCMSCs and sPL mixture was accomplished through a three-way stopcock device, connecting a syringe with sPL or UCMSCs on one side and another syringe with extracellular matrix (ECM) on the opposite side. Thin endometrium models were induced by infusing absolute ethyl alcohol into the uteri of female Sprague-Dawley (SD) rats. Rats were randomly assigned to several groups (Normal, Model, ECM + UCMSCs, ECM + sPL and UCMSCs) and treated for 21 days. Histopathological structures and endometrial thickness were observed using hematoxylin-eosin (HE) staining. ELISA was used to detect PDGF-BB, TGF-β1, E2 and FSH expression levels in serum.

Furthermore, Western blot and immunohistochemical staining were used to assess the expression levels of cyclin D1, CD34, pan-keratin, cytokeratin 18, and vimentin in uterine tissue. HE staining revealed improvements in endometrial thickness, gland number, and blood vessels following treatment with UCMSCs and sPL + UCMSCs in the thin endometrium rat model at 21 days. Immunohistochemical staining indicated decreased cyclin D1, CD34, Pan-keratin, cytokeratin 18, and vimentin expression levels in the model group, which were significantly increased by sPL perfusion or UCMSCs transplantation. Based on Western blot analysis, the experimental group showed significantly higher cytokeratin and vimentin expression than the normal group. Compared with the model group, ELISA results demonstrated that the levels of the PDGF-BB, TGF-β1, E2 and FSH serum in treatment groups returned to normal levels. Compared with the model group, the thickness of the endometrium and the number of glands in sPL or UCMSC treatment groups were significantly higher, and the interstitial blood vessels were denser. UCMSCs and sPL offers significant benefits for thin endometrium treatments. Despite ongoing challenges and difficulties, UCMSCs and sPL shows significant promise for future clinical trials and could provide an alternative to PRP-based treatments. Furthermore, these promising results may contribute to the development of innovative therapeutic strategies for conditions associated with endometrial dysfunction. The combination of sPL and UCMSCs emerges as a potential candidate for clinical interventions aimed at enhancing endometrial regeneration and improving reproductive outcomes. UCMSCs combined with sPL therapy may be a promising approach for treating thin endometrium. However, the future application of this technology should be followed by a more comprehensive investigation.

【关键字】Thin endometrium; Umbilical cord mesenchymal stem cells (UCMSCs); Super activated platelet lysate (sPL); Endometrium regeneration

鼠李糖乳杆菌 SHA113 通过调节肠道干细胞 LGR5 表达缓解结直肠癌进程

庞冰[1]、王岩[1*]

【摘要】肠道干细胞的异常与结直肠癌（CRC）的发生发展密切相关，其中 Lgr5 作为肠道干细胞的重要标志物，成为本研究的重点。益生菌在维持肠道干细胞的正常生理功能、促进肠道健康方面发挥着重要作用。对益生菌与肠道干细胞关系的深入研究，有望为肠道相关疾病的治疗和预防提供新的策略和方法。

本文主要探究自行分离的益生菌 SHA113（LRS）对肠道干细胞 LGR5 的调节进而缓解结直肠癌进程。使用 AOM/DSS 构建小鼠 CRC 模型，使用 LRS 进行干预，转录组测序结果显示 LRS 干预能够显著下调 LGR5 表达。免疫组化结果显示 LRS 干预组 LGR5 的表达水平显著低于模型组。利用 Western blot 技术检测了与细胞增殖、凋亡和转移相关的关键信号通路蛋白。结果发现，模型组β-catenin 的核内聚集明显，下游靶基因如 c-Myc 和 Cyclin D1 显著上调体内实验中，将不同过表达 LGR5 结直肠癌细胞系接种到裸鼠体内，使用 LRS 进行干预，观察肿瘤的生长和转移情况。结果显示 LRS 干预组的肿瘤生长速度更慢，转移灶更少。

综上所述，本实验证实了益生菌 SHA113 能够调节肠道干细胞标志物 Lgr5 在结直肠癌中的表达，抑制激活 Wnt/β-catenin 信号通路。这些发现为结直肠癌的诊断和治疗提供了新的治疗方式。

一种适用于优化低免疫原性干细胞制品的 CD47 突变体

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【摘要】CD47 激活髓系与 NK 细胞表面的免疫抑制受体 SIRPα从而抑制巨噬细胞的吞噬能力、树突细胞的抗原摄取与活化（进而抑制 T 细胞活化），及 NK 细胞功能。

以自体及异体干细胞为代表的细胞制品为基于特定细胞的修复治疗提供了细胞药物来源。其中，同种异体来源的细胞制品具有制备迅速、质量稳定及成本更低等优势，然而受者对异体细胞的免疫排斥反应阻碍了其应用的普及。研究表明过表达 CD47 有效降低主要组织相容性复合体基因失活异体细胞的免疫原性，使其在无需免疫抑制处理的宿主体内存活。然而由于 CD47 信号的多种不利影响，过表达野生型 CD47 对细胞制品的生存能力与功能存在抑制效应。

为去除 CD47 在移植领域的负面功能保留其有益功能，本研究删除了 CD47 的负面信号传导序列，同时将其参与抑制免疫反应的有益功能区段与糖基磷脂酰肌醇膜锚定结构（GPI）连接信号融合表达使其能够正确定位并有效发挥功能。本研究在体内外水平通过凋亡诱导实验、巨噬细胞吞噬实验、肿瘤、干细胞移植实验、血管生成检测实验等确证了该突变体去除了野生型 CD47 介导和加剧移植物损伤及抑制血管新生等负面功能，保留了其保护移植物免受髓系细胞介导的移植排斥等有益功能且其保护水平与野生型 CD47 相当，同时通过造血干/祖细胞移植实验确证了其过表达不影响干细胞功能[1]。因此，该突变体适合应用于优化低免疫原性的转基因细胞产品制备方案。该突变体在异种移植供体转基因动物育种改良方面的应用也将有助于提高移植物的存活率并保护其功能。

【关键字】CD47；SIRPα；低免疫原性干细胞；免疫排斥；异种移植

A second-generation M1-polarized CAR macrophage with antitumor efficacy

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【摘要】Chimeric antigen receptor (CAR) T cell therapies have successfully treated hematological malignancies. Macrophages have also gained attention as an immunotherapy owing to their immunomodulatory capacity and ability to infltrate solid tumors and phagocytize tumor cells. The frst-generation CD3ζ-based CAR-macrophages could phagocytose tumor cells in an antigen-dependent manner. Here we engineered induced pluripotent stem cell-derived macrophages (iMACs) with toll-like receptor 4 intracellular toll/IL-1R (TIR) domain-containing CARs resulting in a markedly enhanced antitumor efect over frst-generation CAR-macrophages. Moreover, the design of a tandem CD3ζ-TIR dual signaling CAR endows iMACs with both target engulfment capacity and antigen-dependent M1 polarization and M2 resistance in a nuclear factor kappa B (NF-κB)-dependent manner, as well as the capacity to modulate the tumor microenvironment. We also outline a mechanism of tumor cell elimination by CAR-induced eferocytosis against tumor cell apoptotic bodies. Taken together, we provide a second-generation CAR-iMAC with an ability for orthogonal phagocytosis and polarization and superior antitumor functions in treating solid tumors relative to frst-generation CAR-macrophages.

All-RNA-mediated targeted gene integration in mammalian cells with rationally engineered R2 retrotransposons

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【摘要】All-RNA-mediated targeted gene integration methods, rendering reduced immunogenicity, effective deliverability with non-viral vehicles, and a low risk of random mutagenesis, are urgently needed for next-generation gene addition technologies. Naturally occurring R2 retrotransposons hold promise in this context due to their site-specific integration profile. Here, we systematically analyzed the biodiversity of R2 elements and screened several R2 orthologs capable of full-length gene insertion in mammalian cells. Robust R2 system gene integration efficiency was attained using combined donor RNA and protein engineering. Importantly, the all-RNA-delivered engineered R2 system showed effective integration activity, with efficiency over 60% in mouse embryos. Unbiased high-throughput sequencing demonstrated that the engineered R2 system exhibited high on-target integration specificity (99%). In conclusion, our study provides engineered R2 tools for applications based on hit-and-run targeted DNA integration and insights for further optimization of retrotransposon systems.

【关键字】Gene editing, gene integration, retrotransposon, R2 element, engineering

VAMP5 is an intrinsic defense factor for embryonic stem cells against SARS-CoV-2 infection

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【摘要】As the key resources for growth and regeneration, stem cells are essential for tissue maintenance and repair. Without any immune system protection, except for the protection from maternal origin, the embryo at the early development stages are often at risk of viral infection, potentially leading to pregnancy loss or vertical transmission during pregnancy. The embryonic stem cells (ESCs) must evolve their own protection mechanisms against viral infection. However, the extent to which ESCs combat SARS-CoV-2 infection, as well as other viruses, has not been fully investigated. Characterizing their activity will shed light on how cell-autonomous antiviral defense to protect ESCs from pathogens and provide the basis for the design and development of broad-spectrum antiviral treatments. Here we identified vesicle-associated membrane protein 5 (VAMP5) as a potent cell-autonomous defense factor against SARS-CoV-2 infection, with high expression levels observed in ESCs and mesoderm. VAMP5 not only shows functional conservation in restricting the replication of SARS-CoV-2 and its variants, as well as other highly pathogenic coronaviruses, but also exhibits effectiveness against the replication of other virus families. This contrasts the concept that resistance escape barriers are higher when relying on host restrictive factors compared to targeting neutralizing antibodies and the virus directly. This will enable us to understand different mechanisms of stem cells resistance to viral infection and also aid in developing entirely new theory to treat viral infection. Remarkably, VAMP5 plays a crucial role in maintaining the stemness of ESCs and is essential for their differentiation. Mechanistic studies revealed that VAMP5 locates to the double membrane vesicles (DMVs) and restricts viral replication relying on its vesicle side of C-terminal domain to interact with viral non-structural protein 8 (NSP8), thereby inhibiting the synthesis of negative strand RNA. These findings suggest that VAMP5 in ESCs disrupts the protected environment of DMVs required for viral genome replication and interacts with RNA replication complexes to defense against viral infection. In addition, this discovery positions VAMP5 as a potential target for designing broad spectrum antiviral treatment capable of inhibiting a broad range of viral infection.

五、组织干细胞

Rapamycin Delays Ovarian Aging and Promoting Stem Cell Function in Somatic Organs

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【摘要】Ovarian function deterioration with age occurs faster in women and this is proposed to link to accelerated somatic aging in other organs, shown as age-associated chronic diseases such as evidently endocrine dysfunction, osteoporosis, breast or ovarian tumor and cardiovascular diseases. Stem cell (SCs) are essential for tissue homeostasis and regeneration, and SCs depletion leads to somatic organ aging. Due to lack of germline stem cells, the ovaries age faster than do most somatic organs that contain SCs. Rapamycin as a mTOR inhibitor was originally utilized as immune-suppressor and subsequently found to protect against aging in multiple species. It remains elusive whether short-term treatment with rapamycin can immediately alleviate ovarian senescence and influence somatic stem cell functions. We conducted short-term rapamycin treatment by one month on reproductively aging (10-month-old C57/BL) mice. The results showed that rapamycin inhibits mTOR and ribosomal translation, delays fibrosis in multiple organs, and reduces the expression of senescence markers and SASP. Notably, rapamycin mitigates meiosis defects in oocytes and cumulus cell senescence, thus improving ovarian function and oocyte quality in aged mice. Meanwhile, our findings demonstrate that rapamycin treatment decrease the expression of senescence markers in stem cells. Rapamycin improves the number and function of somatic stem cells in multiple organs including lungs, muscles, and intestines during treatment. Collectively, short-term treatment with rapamycin both delays the aging of the reproductive system and somatic organs in mammals, highlighting its potential as an anti-aging strategy to rejuvenate stem cell function. Thus, it provides valuable resources and foundation for further evaluation the anti-aging ability of rapamycin in reproductive and tissue stem cells.

【关键字】Rapamycin; Ribosomal translation; Ovarian function; Somatic stem cells; Aging

人胚胎干细胞衍生角膜内皮细胞治疗角膜内膜功能障碍

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【摘要】**Background** Research on human pluripotent stem cells (hPSCs) has shown tremendous progress in cell-based regenerative medicine. Corneal endothelial dysfunction is associated with the loss and degeneration of corneal endothelial cells (CECs), rendering cell replacement a promising therapeutic strategy. However, comprehensive preclinical assessments of hPSC-derived CECs for this cell therapy remain a challenge.

Results Here we defined an adapted differentiation protocol to generate induced corneal endothelial cells (iCECs) consistently and efficiently from clinical-grade human embryonic stem cells (hESCs) with xeno-free medium and manufactured cryopreserved iCECs. Cells express high levels of typical CECs markers and exhibit transendothelial potential properties in vitro typical of iCECs. After rigorous quality control measures, cells meeting all release criteria were available for in vivo studies. We found that there was no overgrowth or tumorigenicity of grafts in immunodeficient mice. After grafting into rabbit models, the surviving iCECs ameliorated edema and recovered corneal opacity.

Conclusions Our work provides an efficient approach for generating iCECs and demonstrates the safety and efficacy of iCECs in disease modeling. Therefore, clinical-grade iCECs are a reliable source for future clinical treatment of corneal endothelial dysfunction.

【关键字】Human embryonic stem cells (hESCs), Induced corneal endothelial cells (iCECs), Corneal endothelial dysfunction, Cell therapy

Dysfunction in neuro-mesenchymal units impairs the development of bone marrow B cells in mice with anxiety

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【摘要】The reduction in B lymphocytes observed in individuals with anxiety disorders may compromise antiviral responses, yet the precise mechanisms behind this decline remain unclear. While elevated glucocorticoid levels have been suggested as contributing factors, anxiety disorders are associated with diminished glucocorticoid signaling.

Given that autonomic nervous system dysfunction is a hallmark of anxiety disorders, we created a mouse model of anxiety disorders by stimulating C1 neurons in the rostral ventrolateral medulla. Through this model, we delved into the impact of ANS dysfunction on adaptive immunity, with a specific focus on the development of B cells and the underlying mechanisms. Additionally, we conducted preliminary investigations into the role of B lymphocytes in the context of anxiety disorders.

We observed that stimulating C1 neurons within the rostral ventrolateral medulla (RVLM) induced psychological stress in mice, resulting in anxiety-like behavior. Using this model, we validated that the sustained activation of sympathetic nerves can disrupt adaptive immunity, particularly impacting the development of B cells. In detail, these mice showed a significant reduction in the quantity and presence of peripheral CD19+ B cells. Disruptions were also observed in all B cell subsets in the spleen of these mice, including germinal center cells (CD19+CD38+CL7+Fas+), plasma cells (CD19+CD38+), follicular B cells (B220+CD21lowCD23+), and marginal zone B cells

(B220+CD21+CD23low). Subsequent studies revealed significantly reduced numbers and frequencies of pre-pro-B-cell precursors (B220+CD19-), immature B cells (B220+CD19+IgM+IgD-), mature B cells (B220+CD19+IgM+IgD+), and plasma B cells (CD19+CD138+) within the bone marrow of these mice.

The underlying mechanism appears to involve the control of B cell development through neuro-mesenchymal units within the bone marrow, with mesenchyme-derived Cxcl12 playing a pivotal role in this regulatory process. The binding of norepinephrine to β3-ADR on MSCs of the bone marrow results in decreased nuclear Sp1 transcription factor content, ultimately leading to the downregulation of Cxcl12. This downregulation, in turn, influences the development of leukocytes.

As the result of rescue of B cell development, bone marrow Cxcl12 injection significantly recovered the number and percentage of B cells in spleen and peripheral blood of mice. Intriguingly, targeting these neuro-mesenchymal units not only restored B cell development but also ameliorated anxiety-like behavior in the mice. The rescue group showed a significant increase in the central area time percent and duration during the Open Field Test (OFT), with no significant differences in general activity. Similarly, rescue group exhibited markedly increase bouts, time, and time percent spent in the open area of the elevated plus maze (EPM).

Our study provides compelling evidence regarding the regulatory role of neuro-mesenchymal units in the development of B cells within the bone marrow, primarily mediated through the norepinephrine-Cxcl12 axis. Additionally, our findings suggest that anxiety disorders can create a vicious cycle, perpetuating ongoing mental and immunological damage and ultimately leading to irreversible harm. To break this cycle, it is essential to focus on the dysfunction of immune cells and strive to restore immune homeostasis in individuals suffering from anxiety disorders.

【关键字】neuro-mesenchymal units / anxiety / B cells development

睾丸间质干细胞通过分泌 osteopontin 促进精子发生

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【摘要】Spermatogonial stem cells (SSC) are of vital importance in maintaining lifelong spermatogenesis and are fundamental to male fertility. The fundamental process of spermatogenesis hinges on the intricate equilibrium between self-renewal and differentiation of SSC. Mesenchymal stromal cells (MSC) play a pivotal role in maintaining tissue homeostasis and stem cell niches, specifically, stem Leydig cells (SLC), which are MSC found within the testis. Our previous studies have demonstrated that transplanted SLC partially differentiate into mature Leydig cells (LC). However, a majority of transplanted SLC remain undifferentiated. It remains unknown whether SLC directly influence SSC or spermatogenesis. Here, we observed close physical proximity between Nestin+ SLC and SSC and found that ablating SLC populations in adult testes resulted in reduced numbers of spermatogonia along with disrupted spermatogenesis and diminished sperm quality. Conversely, transplantation of SLC populations successfully restored spermatogenesis following chemotherapy-induced testicular injury while also alleviating degenerated functions associated with aging mice's sperm production capabilities. Furthermore, our data demonstrated that SLC could promote SSC division and differentiation through a SLC-SSC coculture system. Single-cell RNA sequencing (scRNA-seq) analysis further identified growth factors derived from SLC, such as osteopontin, as potential key regulators of the SSC niche. Together, our finding suggested SLC plays a pivotal role within the spermatogonial niche, and our study would shed new light on male fertility therapy.

基于神经辐射场 3D 打印构建类脊髓组织修复脊髓损伤的研究

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【摘要】背景：脊髓损伤（SCI）是一种极具致残性和破坏性的中枢神经系统损伤，导致不同程度的感觉和运动障碍，严重影响患者的日常生活。功能性神经再生支架治疗 SCI 是现今的研究热点，但标准化支架难以适应人类个体损伤差异，影响 SCI 修复效果。神经辐射场（NeRF)是一种基于深度学习的三维重建技术，能够根据 SCI 损伤区域的二维图像生成三维模型，具备高精度、高效率的优势。本研究基于 SCI 组织工程支架的个性化问题，拟结合 NeRF 和 3D 打印技术，构建定制化的“类脊髓组织”，为 SCI 治疗提供新思路。方法：拟筛选适合负载神经干细胞（NSCs）的 3D 打印材料并进行表征检测；构建并优化 NeRF 架构，对 SD 大鼠脊髓的二维图像进行 3D 重建；通过 3D 打印建立负载 NSCs 和神经营养因子 3 的类脊髓组织；检测类脊髓组织在体外、体内的安全性和有效性。结果：成功构建 NeRF 架构，基于大鼠脊髓的二维图像生成 3D 模型；CCK-8 实验和 Live/dead 染色结果显示甲基丙烯酰化明胶对 NSCs 具备较高的相容性；在体外实验中，Western-blot 和 QPCR 实验结果显示二维培养、三维培养的类脊髓组织均能有效抑制 GFAP 的表达，促进 TUJ-1 的表达，即抑制瘢痕增长和促进神经再生，且三维培养的类脊髓组织体外有效性更好。结论：本研究结合 NeRF 和 3D 打印构建了定制化的类脊髓组织，并在体外验证了细胞相容性和修复脊髓损伤的有效性。

【关键字】脊髓损伤；神经辐射场；3D 打印；神经干细胞

HOXA5 通过 PTPRZ1 介导胶质瘤干细胞增殖影响疾病进展

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【摘要】Glioblastoma is the most malignant glioma among all astrocytic tumors, with a very fast growth rate. 70% to 80% of patients have a disease course of 3 to 6 months, and only 10% have a disease course exceeding 1 year. After active treatment, it is also highly likely to recur and progress rapidly within six months to a year. Therefore, curbing the recurrence of glioblastoma is an important challenge in reducing its mortality rate. GSCs are defined functionally by their ability to self-renewal and differentiate cells, as well as their ability to maintain tumor heterogeneity, tumor growth, and treatment resistance. GSCs have self-renewal and cell differentiation abilities, maintaining tumor heterogeneity, tumor growth, and treatment resistance. Elucidating the molecular mechanisms of GSC regulation will broaden our understanding of this disease and provide insights into effective therapeutic strategies targeting glioblastoma (GBM). In this study, we identify the gene amplification and protein overexpression of HOXA5 in GSCs and its function in regulating GSC maintenance and the downstream transcriptional effector, to explore the significance of HOXA5 amplification/overexpression for GSC identification and prognostic determination. Specifically, HOXA5 gene amplification and the resultant protein overexpression are correlated with increased proportions of GSCs and enhanced self- renewal/invasiveness of these cells. Disruption of HOXA5 expression impairs GSC survival and GBM tumor propagation. Mechanistically, HOXA5 directly binds to the promoter region of protein tyrosine phosphatase receptor type Z1 (PTPRZ1), thereby upregulating this gene for GSC maintenance. Suppression of PTPRZ1 largely compromises the pro-tumoral effect of HOXA5 on GSCs. In summary, HOXA5 amplification serves as a genetic biomarker for predicting worse GBM outcome, by enhancing PTPRZ1-mediated GSC survival.

成年海马神经干细胞调控认知和情绪行为

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【摘要】Adult hippocampal neurogenesis (AHN) is functionally implicated in behavioral responses to stress and antidepressants. However, how the AHN regulates information processing to directly performance behavioral functions is unknown. Here we show in mice that ablation of AHN directly leads to impairments in cognitive, emotional, and social behaviors. The AHN exhibits partial functional heterogeneous along its dorsal-ventral axis, demonstrated by findings that cognitive flexibility is impaired only when the AHN is ablated in the dorsal dentate gyrus (dDG), and avoidance behavior is affected only following the ablation in the ventral dentate gyrus (vDG). In contrast, the functional homogeneous is demonstrated by findings that the ablation of AHN leads to impairments in spatial cue learning, object recognition, and social individual recognition both in dDG and vDG. By monitoring the expression of activity-regulated gene and in vivo calcium signaling, we show that hippocampal subregion outside the neurogenic niche and some extra-hippocampal regions lose their typical activity when responding to anxiety or social contexts. These effects on avoidance are sufficient to confer resilience against anxiety and social withdrawal triggered by acute systemic lipopolysaccharide (LPS) challenge. Our findings further indicate that acute chemogenetic inhibibition of adult born neurons in the vDG is not enough to alter behavior, whereas acute activation of these new neurons in the vDG effectively counteracts the anxiety-like behavior and social withdrawal induced by LPS. Our results suggest that the AHN directly conducts performance in cognitive, emotional, and social behaviors, and may be a key factor in determining the therapeutic effects of neurodegenerative and psychiatric disorder.

鸡胚胎干细胞无饲养层培养体系的建立

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【摘要】目的：本试验利用条件培养基（conditional medium，CM）和基质胶 Metrigel 包被的细胞培养板，成功分离培养出鸡胚胎干细胞（chicken embryonic stem cells，cESCs），利用形态学、碱性磷酸酶（alkaline phosphatase，AKP）染色和胚胎特异性表面抗原 1（stage specific embryonic antigen 1，SSEA-1）鉴定后，细胞状态良好，未分化并保持其多能性，表明本研究建立了 cESCs 无饲养层培养体系，摆脱了培养 cESCs 使用饲养层细胞的束缚，为后续研究提供种子资源。

材料方法：参照本人早前发表文献方法，分离 cESCs，将 cESCs 沉淀用条件培养基重悬，接种至 Matrigel 包被的 24 孔板上，继续培养、换液，5～6 d 后采用全消化法传代，方法如下：加入 PBS 后于 37 ℃孵育 8～10 min，弃掉，0.25% Tripsin 37 ℃消化 5 min，然后于室温下不断吹打 2 min，1000 rpm 离心 10 min，沉淀用条件培养基悬浮。检测 AKP 活性，鉴定 SSEA-1。

结果：cESCs 体积较小，排列紧密，细胞间的界限较清晰，有一个或多个明显的细胞核，卵黄颗粒在原代细胞中含量较多，但会通过细胞传代次数的增加而逐渐减少。可以连续传代且不分化（图 1）。与生长在饲养层上的 cESCs 细胞无明显差别。未分化的 cESCs，AKP 染色后呈阳性，被染成蓝紫色，提示细胞状态良好。SSEA-1 免疫荧光鉴定，镜下可见绿色荧光，结果表明细胞未分化，且保持发育的多能性。

讨论：本研究利用 CM 和 Metrigel 包被的细胞培养板成功建立 cESCs 无饲养层培养体系，cESCs 可体外连续培养，第 3 代 cESCs，状态良好，经冻存和复苏后，细胞活率与饲养层培养的 cESCs 相差不大，且继续培养的 cESCs 分化较少。