2021 CSSCR

干细胞研究与转化 指南粤

中国干细胞第十一届年会

The 11th Annual Meeting of CSSCR

论文汇编

COMPILATION OF PAPERS

2021
12.12-12.15

广州 Guangzhou

主办单位：中国细胞生物学学会干细胞生物学分会

承办单位：中山大学中山眼科中心
眼科学国家重点实验室
干细胞与组织工程教育部重点实验室（中山大学）

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曾 艺 简 介

曾艺，研究员，博士生导师。2005 年毕业于加拿大 Simon Fraser 大学，获分子及细胞生物学博士学位。2005 年至 2010 年在美国 Stanford 大学从事博士后研究。2010 年起任中科院上海生科院生化与细胞所研究员。获国家“杰出青年基金”、中组部“中青年科技创新领军人才”、上海市“优秀学科带头人”、谈家桢“生命科学创新奖”。长期研究成体干细胞命运决定的调控机制，在发现新的成体干细胞的身份、建立成体干细胞的体外扩增体系、发现干细胞微环境因子方面取得一系列国际领先的研究成果。在 Nature、Cell、Cell Stem Cell 等著名学术期刊上发表论文 30 余篇。担任 eLife 期刊编辑及 Development、JBC 编委, 中国干细胞生物学会理事。

Procr 成体干细胞与类器官

曾艺
中国科学院分子细胞科学卓越创新中心

【**Abstract**】It has generally proven challenging to produce functional β cells in vitro. Our recent study uncovers a novel Procr cell population in adult mouse pancreatic islets. The cells do not express differentiation markers and feature epithelial-to-mesenchymal transition (EMT) characteristics. By genetic lineage tracing, Procr islet cells undergo clonal expansion and generate all four endocrine cell types during adult homeostasis. Sorted Procr cells, representing ~1% of islet cells, can robustly form islet-like organoids when cultured at clonal density. Exponential expansion can be maintained over long time periods by serial passaging, while differentiation can be induced at any time point in culture. b cells dominate in differentiated islet organoids, while α, δ and PP cells occur at lower frequencies. The organoids are glucose-responsive and insulin-secreting. Upon transplantation in diabetic mice, the organoids reverse disease. These findings demonstrate that the adult pancreatic islet contains a population of Procr progenitors. We will also describe the physiological relevance of Procr progenitors during postnatal islet development and homeostasis.

王金勇简介

王金勇，中国科学院动物所、北京干细胞与再生医学研究院研究员。长期致力于血液/免疫谱系再生研究，基于“体外再生造血种子、体内发育成熟”两步法策略和细胞谱系命运“关口前移干预”理论，实现 T、B、NK 等单一或多谱系免疫细胞在动物模型移植和疾病模型治疗。近几年在 Nature Immunology, Cell Research, Leukemia, Hematologica 等刊物发表代表性论文。

血液/免疫谱系再生

王金勇
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【摘要】干细胞体外分化获得免疫细胞，在可精确基因编辑、现货式、通用性等方面体现出巨大优势，部分细胞类型在治疗恶性肿瘤方面已经推进到临床试验阶段，健康意义重大。基于造血/免疫发育与调控理论，我们课题组尝试采用了“体外诱导造血/免疫种子，体内发育成熟”的两步法策略来研究获得可移植的造血/免疫种子技术。基于干细胞分化与细胞命运决定理论，我们采用“关口前移干预”策略，筛选能够指导干细胞定向分化为特定造血/免疫谱系的内在转录因子组合。实践中，我们发现：Runx1 和 Hoxa9 两个转录因子的协同表达，可以诱导干细胞定向分化为具有 T 免疫谱系潜能的造血种子细胞；Lhx2, Hoxa9, Runx1 三个因子的组合表达，可以诱导干细胞定向分化为具有 B 免疫谱系潜能的造血种子细胞；Runx1 和 Hoxa10 的组合，可以促进单核/粒髓系再生。上述不同组合诱导产生的造血种子，移植免疫缺陷鼠，均能够在体内生理环境下继续发育成熟，产生谱系对应的终末免疫细胞。体内再生的 T 免疫谱系，包括 CD4SP, CD8SP 成熟亚群，具备完备的生理与免疫功能。再生的 B 免疫谱系，包括所有 B1 和 B2 细胞亚群，在特定抗原免疫后产生特异性抗体、能够形成免疫记忆。在多批次动物实验中，上述再生的免疫种子细胞体现出良好的可移植重复性，嵌合率高，未见致癌性。可以预期，在造血干细胞再生技术尚未突破之前，移植干细胞再生来源的免疫种子细胞，有望成为临床治疗某些免疫缺陷和遗传性疾病的重要手段。

葛 坚 简 介

葛坚，主要研究领域：眼科干细胞与组织工程 ；近视防控研究；青光眼防治研究。相继获得了 973 计划（首席科学家）、863 重大专项、国家自然科学基金重点项目、教育部科学技术研究重大项目等多项基金的资助， 以第一完成人获得国家科技进步二等奖、教育部及广东省科技进步一等奖等奖项十余次。单独或与他人合作发表论文 376 篇， 其中 SCI 收录论文 200 篇。主编卫生部统编八年制《眼科学》 等专著和教材 10 余本。

视网膜类器官构建与移植的进展和挑战

葛坚
中山大学中山眼科中心

【摘要】常见致盲眼病可分为光学性、神经性致盲眼病两大类，光学性致盲眼病可以复明，神经性致盲眼病是不可逆的，全球眼科学家与科学家致力于视神经损伤与修复的研究，视网膜类器官的研究是近年来人类器官再生医学的前沿及热点。类器官是一种在体外由干细胞诱导分化形成的“与体内器官具有高度相似组织结构的简化版器官”，其中视网膜类器官研究深入且广泛。视网膜类器官的诱导分化技术经历了多次改进，效率不断提高，发育程度不断完善。视网膜类器官在作为视网膜发育和疾病模型替代治疗的种子细胞库等方面有着广阔的应用前景。本文就视网膜类器官的构建、移植及移植后存在的问题进行了研究和探讨，并提出了解决途径，如多组学研究、视网膜类器官的标准化、精细化诱导分化系统、与材料学及组织工程学的融合、影像学及功能学检查技术的发展以及类器官移植方式、靶向移植技术及移植部位的改进，以期促进视网膜类器官技术的临床应用。

李劲松 简 介

李劲松博士从事干细胞与胚胎发育相关研究。1993 年毕业于江西农业大学，获学士学位；1996 年毕业于扬州大学，获硕士学位；2002 年毕业于动物研究所，获博士学位；2002 年至 2007 年在洛克菲勒大学从事博士后研究；2007 年 8 月起任生化与细胞所研究员。率领团队建立了小鼠孤雄单倍体胚胎干细胞（即“类精子干细胞”），证明其能代替精子使卵子受精产生健康小鼠（即“半克隆技术”），并利用类精子干细胞携带 CRIPSR-Cas9 文库实现了小鼠个体水平的遗传筛选；提出并推动基于类精子干细胞技术的基因组标签计划。研究成果 2011 年和 2012 年入选“中国科学十大进展”。 以第一作者或通讯作者身份在 Cell, Nature, Cell Stem Cell, Nature Cell Biology 等杂志发表 60 余篇研究论文 。荣获中科院“百人计划”、国家杰出青年科学基金、中青年科技创新领军人才、国家百千万人才工程、中组部万人计划。

类精子干细胞介导的遗传改造

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细胞生物学国家重点实验室，主任

【摘要】哺乳动物单倍体胚胎干细胞是从单倍体囊胚中建立的细胞系，因为只含有一套遗传物质，为在细胞中开展高通量正反向遗传筛选提供了新的工具。另外，携带精子遗传物质的孤雄单倍体干细胞可以替代精子通过卵子注射高效产生半克隆小鼠（因此又称为类精子干细胞），可作为载体将基因编辑器通过“受精”带到胚胎中，为研究胚胎发育和细胞命运决定提供新的遗传学工具。与 CRISPR-Cas9 技术结合，类精子干细胞介导的半克隆技术可以实现：（1）一步获得携带多基因突变的杂合小鼠模型，用于模拟人类多基因介导的复杂疾病；（2）快速获得携带人类疾病相关点突变小鼠用于研究疾病发生的分子机制；（3）一步获得针对不同基因的突变小鼠，实现小鼠个体水平的遗传筛选；（4）一步获得针对特定蛋白质不同碱基突变的小鼠，实现蛋白质关键氨基酸的在体遗传筛选；（5）建立携带蛋白质标签敲入的类精子干细胞库，进而获得携带蛋白质标签的小鼠库，为实现全基因组蛋白质标签计划（genome tagging project, GTP）提供技术保障；GTP 将为开展蛋白质功能的在体、实时、动态、网络研究提供了新的手段。除了基因层面的改造以外，单倍体干细胞还可以用于染色体层面的改造，为研究染色体结构功能的关系以及染色体进化提供了新的平台。

谭 韬 简 介

谭韬，昆明理工大学灵长类转化医学学院教授，博士生导师。主要从事灵长类早期胚胎发育调控研究。相关工作发表在 Science, Cell, Cell Research, Nature Communications, Protein Cell, Cell Discovery, Biology of Reproduction 等学术期刊。曾获中国细胞生物学学会干细胞生物学分会青年研究员奖，并入选国家高层次人才计划青年学者。获国家自然科学基金、科技部重点研发计划等项目支持。现担任中国生物工程学会青年工作委员会委员、中国动物学会生殖生物学分会委员。

灵长类动物胚胎体外培养体系建立与运用

谭韬
四川新健康成生物股有限公司

【摘要】哺乳动物着床后早期胚胎发育，特别是原肠运动时期，受到从转录到转录后水平多层次调控，是生命体发育最重要的事件之一。这一时期的发育异常将导致胎儿畸形等出生缺陷发生，一直以来是领域研究热点与难点，但由于伦理、材料及研究手段的限制对灵长类研究依然是空白。本研究通过建立灵长类胚胎体外长时程培养体系，并结合单细胞转录组学等技术手段，解析了灵长类胚胎着床后特别是原肠运动时期重要的分子与细胞生物学事件,初步阐明了调控胚胎三胚层分化的分子机制。在建立胚胎体外培养系统的基础上，系统评估了人扩展多能性干细胞在食蟹猴中的嵌合能力，回答了异种嵌合细胞如何互作及发育程序差异如何调节等基础科学问题，为解决异种嵌合效率低下等问题提供新的思路。

秦建华简介

秦建华，中科院大连化学物理研究所首席研究员，生物微流控芯片中心主任，辽宁省微流控芯片重点实验室主任；英国皇家化学会 Fellow，国际刊物 Lab on a Chip 副主编,国际刊物 VIEW 顾问编辑；中国生物工程学会生物传感与生物芯片专委会主任；中科院大连化学物理研究所理学博士、加拿大多伦多大学博士后，香港大学理学院、玛丽医院访问学者。已发表 SCI 论文 150 余篇，授权专利 37 项。主要研究方向：微流控器官芯片、类器官及其与生物医学前沿交叉研究。

器官芯片与未来医学

秦建华
中国科学院大连化学物理研究所

【摘要】人体是一个复杂动态的巨系统，涉及多因素、多层次和多维度的集合要素。现阶段，生命科学和医药健康领域的快速发展仍面临一系列严峻挑战，迫切需要对极为复杂的人体器官系统更深层次的理解和认识。当今，生物学与医学、材料、工程和信息等多领域学科的跨界融合，正孕育着新的生命科学与医药健康研究范式，以期从系统视角认识生命本质，阐释疾病发生机理，开发有效应对策略。

器官芯片是新兴前沿交叉领域，融合了物理、化学、生物和工程等多门学科，它可在体外以前所未有的方式见证人体对外界刺激的响应，具有颠覆性技术性质。本报告将从人体复杂器官生理特点出发，以作者团队近几年的系列工作为基础，着重介绍器官芯片起源，工程学策略及其在疾病研究和药物评价等方面的研究进展，并对器官芯片、类器官技术面临的挑战，及其在未来医学的应用前景予以展望。

关键词：器官芯片；类器官；干细胞；疾病模型；药物评价

黄 河 简 介

黄河，浙江大学求是特聘教授，973 首席科学家，主任医师，博士生导师。现任浙江大学医学院附属第一医院院长，浙江大学血液学研究所所长。任中华骨髓库专家委员会副主任委员，中华医学会血液学分会常务委员，亚太国际骨髓移植组织国际学术委员会常务委员，欧洲骨髓移植组织国际学术委员会委员，亚洲细胞治疗组织学术委员会委员等学术职务。主要研究方向为造血干细胞移植的临床和基础研究，干细胞基础及应用研究，免疫治疗临床基础及应用研究。先后于 2003 年及 2015 年 2 次荣获国家科技进步奖二等奖。作为负责人承担 973，863，国家自然科学基金重点项目，国家自然科学基金国际合作与交流项目等 27 项。在 Blood、Leukemia 等 SCI 杂志发表通讯作者论文 192 篇。获省部级以上奖项 14 项，授权发明专利 17 项。在国际大型会议担任主席、特邀报告和口头报告 98 次。作为大会主席分别于 2005 年和 2014 年在杭州主办亚太国际骨髓与造血干细胞移植大会。主编人民卫生出版社全国研究生《血液内科学》教材，参编著作及教材 12 部。任国际造血干细胞移植领域权威杂志 BMT，BBMT, JHO 编委。

CART 治疗与造血干细胞移植的联合应用

黄河
浙江大学医学院附属第一医院

【摘要】背景:嵌合抗原受体 T（CAR-T）治疗能高效地诱导复发难治急性淋巴细胞白血病（r/r B-ALL）患者获得缓解，但患者仍面临再次复发风险。桥接异基因造血干细胞移植作为 CAR-T 治疗 r/r B-ALL 后的巩固治疗是一个具有争议性的治疗策略。目前半相合造血干细胞移植（haplo-HSCT）技术日渐成熟，CAR-T 治疗后桥接 haplo-HSCT 是否能使患者受益，是否有特异性的生物标志物来预测 CAR-T 治疗后桥接 haplo-HSCT 的预后情况，我们进行了一项多中心回顾性研究，以回答上述 2 个关键科学问题。

方法:本研究纳入全国 11 家医疗机构经靶向 CD19 CAR-T 细胞治疗并获得 CR 的复发难治急性淋巴细胞白血病患者，共计 122 例，其中 55 例患者桥接半相合造血干细胞

移植（移植组），67 例患者因个人意愿拒绝桥接半相合造血干细胞移植（未移植组），进入随访观察。

结果:未移植组和移植组患者 2 年无白血病生存（LFS）、2 年总体生存（OS）分别为 32.8%和 65.6%（P<0.001）、36.4%和 77.0%（P<0.001）。多因素分析显示，移植前微小残留病(MRD)阳性是影响 LFS (P=0.005)、OS (P=0.035)和累积复发率(P=0.045)独立危险因素。进一步根据半相合造血干细胞移植前 MRD 状态将移植组患者分为 MRD 阳性组（15 例）和 MRD 阴性组（40 例）；MRD 阳性组和 MRD 阴性组患者 2 年累积复发率、2 年累积非复发死亡率、2 年 LFS、2 年 OS 分别为 65.8%和 17.3%、6.7%和 6.6%、27.6%和 76.1%、62.7%和 83.3%。

结论:CAR-T 细胞疗法桥接半相合造血干细胞移植治疗复发难治急性淋巴细胞白血病是安全有效的治疗策略，经 CAR-T 细胞治疗后获得 MRD 阴性的患者应选择合适时机行异基因造血干细胞移植。

姚红杰简介

姚红杰，2005 年在中国科学院植物研究所获得博士学位。2005 年-2011 年先后在美国宾州州立大学、美国 NIH 从事博士后研究工作。2011 年 10 月至今，中国科学院广州生物医药与健康研究院研究员、博士生导师。2019 年国家“杰青”获得者。研究成果以通讯作者身份发表在 Cell Stem Cell, Sci Adv, Nat Commun (2 篇), NAR 等学术期刊上。

Chromatin modifications and structural dynamics in determining cell fate

姚红杰
中国科学院广州生物医药与健康研究院

【**Abstract**】Chromatin modifications and structure dynamics play important roles in regulating gene expression. Two-dimensional (2D) chromatin structure mainly involves a series of epigenetic modifications and special nucleic acid structure, such as R-loop formed by RNA:DNA hybrid and single strand DNA. Three-dimensional (3D) chromatin structure primarily refers to the high-order chromatin structure, including chromatin loops mediated by structural proteins (such as CTCF, Cohesin and YY1) and topological associated domain and so on. In this talk, I will briefly introduce our recent discoveries on the roles of R-loop and histone H2AK119ub1 in determining cell fate at the 2D level. Furthermore, I will present our unpublished data on the functions of chromatin structural proteins CTCF/YY1 in regulating induced pluripotent stem cells and extended pluripotent stem cells, respectively, at the 3D level.

沈晓骅简介

沈晓骅清华大学医学院教授、长江学者特聘教授。探索非编码基因组影响转录和染色质结构的新模式，从独特视角认识发育和干细胞分化中细胞命运决定的普适性规律。近年成果包括:揭示非编码 RNA 顺式调控邻近转录和染色质构象；揭示基因组折叠的基层规律，提出转座子重复序列（L1 和 B1/Alu）是大尺度染色质三维结构形成的遗传分子基础；RNA 结合蛋白通过相分离反馈调控转录和染色质状态。获国家杰青和求是杰出青年等奖励。

The noncoding genomes in transcription and chromatin regulation

沈晓骅

清华大学

【**Abstract**】Abstract: Much of the developmental complexity and biodiversity of higher eukaryotes is thought to arise from gene regulation. RNA represents a hidden layer of regulatory information in complex organisms. I will discuss our recent progress in exploring fundamental functions of genomic repeats, noncoding RNA, and RNA-binding protein in the regulation of transcription and genome organization.

Jose Silva 简 介

José SilvaJosé received his first degree in Biology from the University of Porto, in Portugal. He joined the GABBA PhD graduate program from University of Porto and then went on to do his PhD studies at Imperial College under the supervision of Professor Neil Brockdorff on heritable silencing mechanisms during mouse development. In his PhD José linked for the first time Polycomb group proteins to histone methylation and to random X-chromosome inactivation. After a post-doc at the University of Edinburgh where José identified the first defined gene with reprogramming activity, Nanog, he started his own laboratory in 2008 at the Wellcome-MRC Cambridge Stem Cell Institute at the University of Cambridge. His work was focused on the investigation of the biology of reprogramming adult cells back into Embryonic stem cells and was sponsored by prestigious Wellcome Trust fellowships. His work has led to numerous scientific contributions in the field of stem cell biology and was published in top tier scientific journals. Recently, José relocated his laboratory to Guangzhou, China, where he is now a Principal Investigator at the Guangzhou Laboratory.

Pluripotency and epigenetic processes

Jose Silva
生物岛实验室

【**Abstract**】Embryonic stem cells (ESCs) are derived from the naïve pluripotent cell population of the pre-implantation embryo and are considered to be its in vitro counterpart. In the past years both human and mouse naïve ESCs have been either captured or converted to culture in the presence of both Mek/Erk and Gsk3b signalling inhibition (2i) together with leukaemia inhibitory factor (LIF). Stat3 signalling activation and modulation of Erk signalling, pathways which are affected by two of the aforementioned molecules, are also instrumental for the establishment of the pluripotent compartment in the early embryo. In addition, there are also reports that these conditions cause global genome DNA demethylation similar to that observed in the naïve epiblast and primordial germ cells (PGCs) during development. However, they also cause imprint erosion and impaired developmental potential of ESCs after prolonged in vitro culture. In my presentation I will discuss that DNA demethylation and epigenetic imprint erasure are linked to pluripotency via the activity of pluripotency associated genes.

丁俊军简介

丁俊军，2009 年 6 月毕业于北京生命科学研究所，获理学博士学位。后于美国西奈山医学院进行博士后研究。研究工作主要集中在胚胎干细胞、体细胞重编程和早期胚胎发育的相分离、表观遗传和染色质高级结构的调控机制，已在国际著名期刊以通讯和第一作者（含共同）身份发表 SCI 学术论文多篇，包括 Nature、Cell Stem Cell（两篇）、Cell Research、Genome Biology 等。

Phase Separation of OCT4 Controls TAD Reorganization to Promote Cell Fate Transitions

丁俊军
中山大学

【**Abstract**】Topological-associated domains (TADs) are thought to be relatively stable across cell types, although some TAD reorganization has been observed during cellular differentiation. However, little is known about the mechanisms through which TAD reorganization affects cell fate or how master transcription factors affect TAD structures during cell fate transitions. Here, we show extensive TAD reorganization during somatic cell reprogramming, which is correlated with gene transcription and changes in cellular identity. Manipulating TAD reorganization promotes reprogramming, and the dynamics of concentrated chromatin loops in OCT4 phase separated condensates contribute to TAD reorganization. Disrupting OCT4 phase separation attenuates TAD reorganization and reprogramming, which can be rescued by fusing an intrinsically disordered region (IDR) to OCT4. We developed an approach termed TAD reorganization-based multiomics analysis (TADMAN), which identified reprogramming regulators. Together, these findings elucidate a role and mechanism of TAD reorganization, regulated by OCT4 phase separation, in cellular reprogramming.

高亚威简介

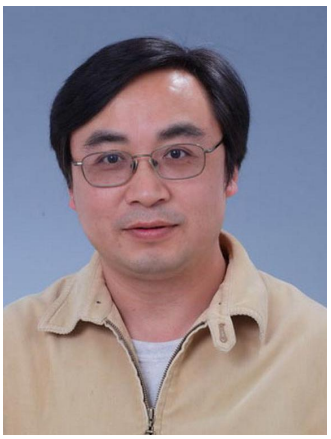
高亚威，同济大学教授，主要从事发育、转分化、核移植和诱导重编程等过程的多层级的表观调控机制研究。建立和完善多种微量测序技术，开展了包括早期胚胎组蛋白修饰图谱的测定和核移植胚胎重编程机制等方面的研究，成果之一发表在 Nature（2016）。主持参与多个 RNA 表观修饰调控的相关课题，成果之一发表在 Science（2020）。以第一作者和通讯作者在 Science, Nature，CSC，NCB 等刊物发表多篇论文。

RNA-chromatin Cross-talk Mediated by m6A on Repeat RNAs in ESCs and Early Embryos

高亚威
同济大学

【 **Abstract** 】 N6-methyladenosine (m6A) on chromosome-associated regulatory RNAs (carRNAs), including repeat RNAs, plays important roles in tuning the chromatin state and transcription, but the intrinsic mechanism remains unclear. Here, We found that METTL3 deposits m6A modifications on chromosome-associated regulatory RNAs (carRNAs), including promoter-associated RNAs, enhancer RNAs, and repeat RNAs. YTHDC1 facilitates the decay of a subset of these m6A-modified RNAs, especially elements of the long interspersed element-1 (LINE1) family, through the nuclear exosome targeting–mediated nuclear degradation. Reducing m6A methylation by METTL3 depletion or site-specific m6A demethylation of selected carRNAs elevates the levels of carRNAs and promotes open chromatin state and downstream transcription. Furtherly we observed indispensable roles of YTHDC1 in the embryonic stem cell (ESC) self-renewal and exiting of 2C-programming, which highly depends on its m6A-binding ability but insensitive to Mettl3. Detailed analyses revealed that YTHDC1 recognizes both Mettl3-sensitive and insensitive m6A on LINE1 RNAs in the nucleus and regulates the formation of the LINE1-NCL partnership and the chromatin recruitment of KAP1. Moreover, the establishment of H3K9me3 on LINE1 scaffold-regulated regions, including 2-cell retrotransposons, is interrupted in Ythdc1-depleted ESCs and inner cell mass (ICM) cells, which consequently increases the transcriptional activity. Our study reveals a new link between m6A and the RNA scaffold, providing a new model for the RNA-chromatin cross-talk.

李磊简介



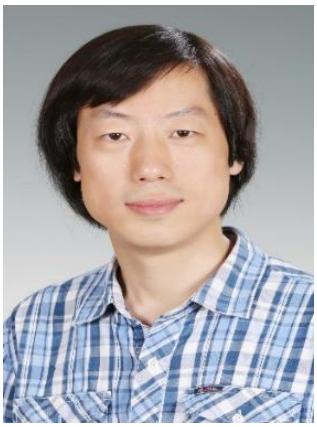
李磊，中国科学院动物研究所干细胞与生殖生物学国家重点实验室研究员。实验室主要敲除小鼠、早期胚胎体外培养系统和多能干细胞分化等模型，研究哺乳动物胚胎发育母源调控、早期细胞谱系形成和配子发生的分子机制。发现哺乳动物第一个母源复合体 SCMC，围绕该复合体进行系列研究；建立了高效的哺乳动物早期原肠胚发育的体外模型；建立了第一个哺乳动物原肠运动即将起始前的多能干细胞系 fPSCs。

Elimination of Naïve Regulation Networks Is Essential for Naïve-to-Formative Pluripotency Transition

李磊
中国科学院动物研究所

【**Abstract**】The transition of mammalian early epiblast at different phases is characterized by the differences of pluripotent states and developmental potential, involving extensive transcriptome changes. However, the role of post-transcriptional RNA degradation modulation in cell fate transition remains largely unexplored. Here, we report that deadenylase Cnot8 of Ccr4-Not complex specifically plays a role in naïve-to-formative transition of pluripotent stem cells (PSCs). Disruption of Cnot8 results in early embryonic lethality, accompanying with increased expression of naïve transcription factors in mouse epiblasts. Cnot8 depletion leads to accumulated expression abundances and increased poly(A) tail lengths of massive transcripts including mRNAs of naïve regulation networks, impairing the naïve-to-formative conversion. Mechanistically, Cnot8 interacts with Tob1 and Pabpc1 to guarantee the prompt mRNA deadenylation and degradation of naïve regulation networks at specific time. Together, these findings delineate the specific role and mechanism of Cnot8 in PSC fate transition through global degradation of mRNAs for naïve regulation networks.

汤富酬简介



汤富酬，北京大学生命科学学院 BIOPIC 中心教授，北京未来基因诊断高精尖创新中心副主任。主要从事人类早期胚胎以及生殖系细胞发育以及癌症的单细胞功能基因组学研究，所发表文章已被引用 11,000 多次。在国际上率先系统发展了单细胞功能基因组学研究体系，开启了单细胞转录组测序时代（发展了世界上第一个单细胞转录组测序技术（2009），第一个单细胞 DNA 甲基化组测序技术（2013），第一个单细胞多组学测序技术（scTrio-seq, 2016），第一个单细胞表观基因组多组学测序技术（scCOOL-seq, 2017），第一个高精度单细胞转录组单分子测序技术（SCAN-seq，2020），第一个单细胞基因组单分子测序技术（SMOOTH-seq，2021））。 在此基础上，发现了人类生殖系细胞发育过程中基因表达网络的多项重要表观遗传学调控机理，以通讯（或者共同通讯）作者身份在 Cell (2013, 2015, 2020)，Nature (2014, 2016, 2018, 2019)，Science (2016, 2018)，Cell Stem Cell (2014, 2017a,b,c, 2018, 2019), Nature Cell Biology (2018a,b), Nature Genetics (2018), Genome research (2013), Gut (2020), Cancer Cell (2020)等期刊发表论文 70 余篇。其中多项研究工作获评 2014 年度和 2015 年度中国科学十大进展，以及 2015 年度和 2019 年度中国生命科学领域十大进展。目前为 Cell Stem Cell，Genome Biology, Genomics Proteomics & Bioinformatics, Open Biology, Precision Clinical Medicine 等学术期刊编委。多次受邀参加 AGBT (Advances in Genome Biology & Technology)、ISSCR (International Society for Stem Cell Research)、ICHG (International Congress of Human Genetics)、Gordon Conferences (Epigenetics series, Germ cell development series, Cancer series)、HCA (Human Cell Atlas)等国际会议并作受邀报告。组织并主持了冷泉港亚洲单细胞基因组学前沿国际会议（2016, 2018, 2020）。

利用单细胞测序技术探索人类早期胚胎发育的奥秘

汤富酬
北京大学生命科学学院

【**Abstract**】Gonadal somatic cells are the main players in gonad development and are important for sex determination and germ cell development. Here, using a time-series

scRNA-seq strategy, we analyzed the fetal germ cells (FGCs) and gonadal somatic cells in human embryos and fetuses. Clustering analysis of testes and ovaries revealed several novel cell subsets, including POU5F1+SPARC+ FGCs and KRT19+ somatic cells. Furthermore, our data indicated that BMP signaling pathway plays cell type-specific and developmental stage-specific roles for testis development, and it promoted the gonocyte-to-spermatogonium transition (GST) process in late testicular mitotic arrest FGCs. Intriguingly, the testosterone synthesis function transitioned from fetal Sertoli cells to adult Leydig cells in a step-wise manner. Moreover, interactions between gonadal somatic cells were systematically explored in our study. More importantly, we identified cell type-specific developmental defects of both FGCs and gonadal somatic cells in a Turner syndrome embryo (45, XO). Our work provides a blueprint of the complex yet highly ordered development and interactions of human FGCs and gonadal microenvironment cells.

汪阳明简介

汪阳明，北京大学教授。2000 年北京大学生物技术学士，2006 年 UIUC 生物化学博士，2006-10 年 UCSF 博士后。主要研究干细胞和非编码 RNA。解析了 miRNA 对胚胎干细胞自我更新的调控机制；发明了 miRNA 激活的 Cas9 技术和新型非编码 RNA 启动子活性报告基因；鉴定了调控 ERK 通路和胚胎干细胞自我更新的长非编码 RNA 以及全能样状态转化的通路 PIAS4-DPPA2。

An Intermediate State during 2C-like to Pluripotent Stem Cell Transition

汪阳明
北京大学分子医学所

【**Abstract**】Mouse embryonic stem cells (ESCs) cycle in and out of 2-cell like state that retains the ability to differentiate into both embryonic and extraembryonic cell lineages. The molecular mechanism that regulates the exit of 2-cell like state remains unclear, partially due to the lack of reporter genes marking the exiting intermediate state. Here we identified an intermediate state during 2-cell like to pluripotent state transition using a novel reporter system. Interestingly, this intermediate state clustered closely to 8-16 cell stage embryos at transcriptome level. Using this reporter system, we uncovered that the pluripotency gene Oct4 drives the exit of 2-cell like state. Furthermore, through a siRNA screening, we identified three other regulators including Chaf1a, Smg7 and Upf1 that promotes the exit of 2-cell like state. Our study shows that the exiting of 2-cell like state mimics early embryonic developmental process and unravels the regulatory mechanism underlying the exit of 2-cell like state. The intermediate state we identified provides a unique resource for studying the transition process from 2-cell like to pluripotent state.

石莉红简介

石莉红，中国医学科学院血液病医院研究员。研究方向为造血谱系分化命运决定及与疾病的关系。在 Cell Stem Cell（2020、 2021）、Nat Med、Dev Cell、Nat Commun、 Adv Sci 等期刊上发表多篇论文。担任中国病理生理学会第九届实验血液学常委，中国生理学会血液学专业委员会委员， 中华医学会青年委员等。荣获国家引进人才青年项目、协和学者特聘教授等荣誉称号。

造血干细胞异质性与血液肿瘤

石莉红
中国医学科学院血液病医院

【摘要】单细胞解析造血干细胞异质性与骨髓增殖性肿瘤发病和治疗的关联

虽然干细胞异质性的概念已广为人知，但其病理意义研究仍知之甚少。研究采取单细胞转录组（RNA-seq）与单细胞 genotyping 联合分析策略（Single-cell RNA-seq coupled with mutation detection），对初诊及治疗的 JAK2V617F++骨髓增殖性肿瘤患者的造血干细胞进行研究。在造血干细胞层面准确区分疾病驱动克隆（突变细胞）和非突变细胞后，研究在初诊 JAK2V617F+原发性血小板增多症患者中发现，由 JAK2 突变驱动、具有巨核细胞（Mk）分化偏好的造血干细胞亚群比例显著扩大，并具有增强的增殖和巨核分化潜能，且这些突变的细胞中具有增强的干扰素信号，并表现出对干扰素信号刺激的高敏性。而在 JAK2V617F+原发性血小板增多症患者的治疗期间，巨核谱系偏好的造血干细胞亚群比例降低。其中治疗后纯合突变和杂合突变造血干细胞行为有所不同：纯合突变造血干细胞表现出静息和休眠的特征，而杂合突变造血干细胞则表现出凋亡的特征；提示现有的治疗手段（干扰素、羟基脲）无法根治，“休眠”的纯合突变细胞可能是骨髓增殖性肿瘤复发根源。该研究表明造血干细胞异质性和骨髓增殖性肿瘤在病理发生和临床治疗方面具有相关性，提示造血干细胞异质性是具有相同起始突变的骨髓增殖性肿瘤产生不同亚型的基础之一，对骨髓增殖性肿瘤发病机制层面提供了新的角度。此外，该研究还提示了现有的治疗手段不能根治该病，联合抗炎药物有可能取得更好的治疗效果。（Tong J. et al., Cell Stem Cell, 2021）

王 莹 简 介

王莹，中科院上海营养与健康研究所研究员，国家“万人计划”青年拔尖，从事干细胞与组织微环境研究。以通讯及第一作者身份在 Cell Metab、Nat Rev Drug Discov、Nat Immunol、Sci Adv、PNAS 等发表论文 36 篇，文章引用次数 >10,000，申请及授权专利 12 项。担任 Cell Immunol 副主编，Cell Death Dis 分审主编， Biol Direct 编委等。

Novel Paracrine Factors from Mesenchymal Stem Cells to Shape Immune Responses

王莹
中国科学院上海生命科学研究院营养与健康研究所

【**Abstract**】 The discovery that mesenchymal stem cells (MSCs) contribute to tissue regeneration by modulating inflammation has revolution-ized stem cell therapy for the treatment of inflammatory diseases. The mechanisms of their therapeutic effects are multifaceted, but in general, the arrival of MSCs in damaged tissues can establish balanced immune status and regenerative microenvironment. This mode for MSC-based tissue regeneration is cell empowerment. The therapeutic effects of MSCs require the existence of certain inflammatory cytokines. However, in the absence of sufficient proinflammatory stimuli or in the presence of anti-inflammatory medications, MSCs are animated to promote immune responses and unable to alleviate inflammatory disorders. The first case reported that exogenously administered MSCs could successfully alleviate refractory acute graft-versus-host disease (aGvHD). However, in a US Food and Drug Administration approved clinical trial, no significant improvement was observed when the data of a large scale MSC based aGvHD trial were analyzed. These conflicting results raise fundamental issues for how to successfully apply MSCs in patients suffering from various types and stages of inflammatory diseases. By deciphering the impact of co-administration of steroids and MSCs on aGVHD, we found that steroids could abolish the therapeutic effect of MSCs on aGvHD and eliminate their suppression on $CD8^+$ T cells. Among various paracrine factors secreted by steroid-treated MSCs, we found that VEGF-C is responsible for the exacerbation of aGVHD. Mice with VEGFR3 conditional deletion in $CD8^+$ T cells demonstrated that VEGFR3 signaling mediates the activation and proliferation of CD8+ T cells. Inhibition of VEGFR3 signaling could improve the efficacy of MSCs in aGvHD, even concomitant with steroid administration. Recently, we identified that MSCs under low oxygen ameliorated autoimmune disease through production of insulin like growth factor 2 (IGF2). IGF2 preprogramed maturing macrophages to acquire oxidative phosphorylation-dependent anti-inflammatory properties. Such regulation acted through IGF2R. We also linked metabolisms to immune cell repair functions. These findings guide us toward therapeutic strategies for managing and improving healing in the clinic.

岳 锐 简 介

岳锐，同济大学长聘教授，生命科学与技术学院副院长，分子与细胞生物学系主任，教育部“细胞干性与命运编辑”前沿科学中心副主任。主要研究方向为骨骼与造血干细胞调控机制。以第一或通讯作者身份在 Cell、Cell Stem Cell、Cell Research、EMBO Journal 和 Cell Reports 等杂志发表论文十余篇。2017 年入选“海外高层次人才”，主持国家重点研发计划项目 1 项，基金委重大研究计划项目 1 项，面上项目 2 项。

骨骼干细胞与骨骼再生

岳锐
同济大学

【摘要】骨骼组织的发育、维持、再生和衰老受到骨骼干细胞（Skeletal Stem Cells, SSCs）的严密调控。目前的观点认为，成体骨骼组织由多种位于不同区域（如骨髓、生长板、骨外膜等）的 SSCs 所共同维持。近年来，我们围绕不同区域 SSCs 的动态调控机制与骨骼再生修复这一重大科学问题开展了系统性研究。我们在骨髓 SSCs 中发现丝氨酸蛋白酶 Fap 是一种新颖的成骨抑制因子。通过遗传学方法敲除 Fap 或利用小分子药物抑制 Fap 活性能够同时促进骨生成并抑制骨吸收，提示 Fap 可能成为新一代抗骨质疏松药物靶点。此外，我们最近的研究在早期人类胚胎长骨和颅顶骨的软骨周发现了一群胚胎骨骼干祖细胞（eSSPCs），其高表达粘附分子 CADM1 以及 FOXP1/2/4 转录调控网络。eSSPCs 可以自我更新并具有较强的成骨和成软骨分化能力，跨物种比较分析发现其在小鼠胚胎中非常保守。我们目前正在进一步探索小鼠胚胎和成体期 eSSPCs 的定位与分化潜能，以期为治疗骨与软骨退行性疾病提供新疗法。

【关键词】

曲 静 简 介

曲静，中科院动物研究所研究员，中国科学院大学存济医学院教授，中国生物物理学会衰老生物学分会副会长，国自然基金委“组织器官再生修复的信息解码及有序调控” 重大研究计划专家组成员。获国自然基金委优青及杰青项目资助，主持重点研发计划“灵长类增龄性相关健康状态减损的生物学基础”项目，国自然基金委重大研究计划重点支持项目。主要研究方向为：灵长类器官及干细胞衰老研究，与结合干细胞及基因编辑技术治疗衰老相关疾病。

Gene Therapy Alleviates Aging Defects by Preventing Cellular Senescence

曲静
中国科学院动物研究所

【摘要】干细胞衰老是机体衰老的重要表现，增强干细胞活力能有效延缓机体衰老。发现并确证调控干细胞衰老的核心因子，可为发展器官衰老及相关疾病的干预手段提供了全新靶标。我们通过基因治疗手段，将人间充质干细胞“年轻”因子导入生理性衰老小鼠或骨关节炎小鼠的关节腔后，可刺激关节软骨的原位再生，改善骨密度减低、软骨损伤、关节炎症等症状。进一步通过 CRISPR/Cas9 全基因组筛选技术，我们鉴定了促衰老基因的系统“名录”，发现其中排名最高的因子 KAT7，作为一种组蛋白乙酰转移酶，可选择性催化组蛋白 H3K14 的乙酰化，促进 p15INK4b 表达，进而诱导人间充质干细胞的衰老。KAT7-p15INK4b 这一通路在不同物种以及不同细胞类型中均发挥保守的衰老促进作用。通过尾静脉注射慢病毒载体，靶向敲低 Kat7 的表达，可有效减少年老小鼠肝脏中衰老细胞的比例，降低血液中促炎因子的水平，改善小鼠的健康状态，延长生理性衰老和早衰症小鼠的寿命，从概念上证明了基于单因子失活的基因治疗策略，有助于延缓器官乃至机体的衰老。

【关键词】

张 亮 简 介

张亮，中国科学院上海营养与健康研究所研究员/研究组长，国家“青年千人计划”和中国科学院“百人计划”A 类入选者。主要致力于皮肤衰老、再生、病变的机制和干预途径研究。2001 年本科毕业于北京大学生物技术系，2008 年博士毕业于美国科罗拉多大学博尔德分校分子细胞及发育生物学系，2008-2014 在美国洛克菲勒大学从事博士后研究，2014 至今任中国科学院上海营养与健康研究所研究员。

造血干细胞的内皮起源

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【摘要】造血干细胞（Hematopoietic stem cell, HSC）可以自我更新并产生所有类型的血液细胞，起源于胚胎发育中期主动脉-性腺-中肾（aorta-gonad-mesonephros, AGM）区背主动脉的生血内皮细胞。HSC 潜能生血内皮细胞的精确捕获较难实现，特化路径中的细胞转归及分子事件仍不清晰；人类早期胚胎造血发育的认识则更加有限；卵黄囊作为重要胚外造血器官，生血内皮的功能和分子特征也有诸多未知。我们的研究首先利用单细胞转录组测序刻画了人类及小鼠胚胎内皮细胞向 HSC 转化过程的细胞及分子事件，揭示了 HSC 生血内皮的动脉内皮身份，筛选出特异富集 HSC 生血内皮的表面标志。利用表面标志组合 PK44（CD41-CD43-CD45-CD31+CD201+Kit+CD44+）以及 Neurl3-EGFP 转基因，我们实现了对小鼠 HSC 生血内皮的高效富集，并揭示了生血内皮细胞的内皮/造血双潜能。我们进一步探究了 PK44 群体的时空和功能异质性，并揭示了卵黄囊中 PK44 细胞的 HSC 潜能。另一方面，结合表征动脉内皮的 Gja5-EGFP 报告基因，我们还探索了胚外卵黄囊动脉内皮细胞的体外生血潜能及体内重建功能。利用 Hlf-tdTomato 报告基因证实，生血内皮细胞必须在表达 CD45 之前获得 Hlf 的表达才能生成 HSC。利用 Hlf-CreER 的谱系示踪发现它标记的胚胎中期造血前体细胞长期稳定地贡献成体骨髓 HSC 池以及平衡型的多系造血。通过对单细胞转录组数据的整合分析，我们揭示了人和小鼠之间 HSC 发育过程中关键细胞群体和分子特征的转录组保守性，并预测人胚新生 HSC 成熟过程与小鼠不同的动力学特征。这一系列关于 HSC 内皮起源的新发现，将为 HSC 体内发育及体外再生研究提供重要的理论基础、数据库资源和小鼠模型。

皮肤上皮组织衰老和再生的干细胞机制及干预

张亮
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【摘要】包括表皮和毛囊等附属物的皮肤上皮组织是人体中少数终生持续再生的组织。这种再生能力在衰老过程中逐渐衰退，导致表皮变薄、愈伤缓慢、毛发再生缺陷等典型的皮肤衰老症状。作为一种高更新率的组织，皮肤上皮组织衰老与其干细胞的衰竭密切相关，但其中的分子机制并不完全清楚，也缺乏靶向干预手段。

我们发现 miR-31 是驱动皮肤毛囊干细胞衰老耗竭的一个关键因子和潜在干预靶点。小鼠和人类毛囊干细胞在衰老过程中均显著上调 miR-31。它在小鼠皮肤上皮组织中的过表达导致皮肤早衰，而其条件性敲除显著抑制皮肤的自然衰老与电离辐射性早衰，且无明显副作用。此外，我们还发现紫外光和物理损伤均可刺激皮肤上皮干细胞迅速上调 miR-31，这与“风吹日晒”加速皮肤衰老的传统观念一致。

机制上，我们发现 miR-31 通过下调其靶基因 Clock 来激活 MAPK/ERK 信号通路，进而驱动毛囊干细胞向表皮异常转分化并耗竭。在此基础上，我们发现 MAPK/ERK 小分子抑制剂（如临床抗癌药物曲美替尼等）能够在小鼠模型中拮抗电离辐射导致的皮肤早衰，显著抑制白发、秃发、愈伤功能障碍等表型。这提供了一种极具临床潜力的皮肤衰老和早衰防治途径。我们还发现这条信号通路在人和小鼠的表皮细胞之间高度保守，而人表皮组织在其自然衰老和电离辐射早衰过程中均显著上调 miR-31。这提示该通路可能同时参与了人毛囊和表皮的衰老过程。

郭伟翔简介

郭伟翔 2008 年获中国科学院动物研究所博士学位，2008-2011 年在美国新墨西哥州大学从事博士后研究， 2011-2014 年在美国威斯康星大学麦迪逊分校从事博士后研究，2014 年任中国科学院遗传发育所研究员。2015 年入选“海外高层次人才引进计划”青年项目。郭伟翔博士着重研究神经干细胞和神经发生的细胞和分子机制，以及它们在神经退行性疾病和神经发育疾病的发生机制，从而为这些疾病的预防和治疗提供理论依据。其研究结果以通讯作者发表在 Cell Stem Cell、Neuron、Mol. Psychiatry、PNAS、J Cell Biol. Cell Rep 等国际主流学术期刊。

Environmental Influence on Adult Neurogenesis

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Adult hippocampal neurogenesis contributes to learning and memory, and is sensitive to a variety of environmental stimuli. Exposure to a hypomagnetic field (HMF) influences the cognitive processes of various animals, from insects to human beings. However, whether HMF exposure affect adult hippocampal neurogenesis and hippocampus-dependent cognitions is still an enigma. Here, we showed that male C57BL/6J mice exposed to HMF by means of near elimination of the geomagnetic field (GMF) exhibit significant impairments of adult hippocampal neurogenesis and hippocampus-dependent learning, which is strongly correlated with a reduction in the content of reactive oxygen species (ROS). However, these deficits seen in HMF-exposed mice could be rescued either by elevating ROS levels through pharmacological inhibition of ROS removal or by returning them back to GMF. Therefore, our results suggest that GMF plays an important role in adult hippocampal neurogenesis through maintaining appropriate endogenous ROS levels.

吴青峰简介

吴青峰，中科院遗传发育所研究员。2005 年毕业于复旦大学获学士学位，2012 年毕业于中科院神经所获博士学位，2012-2016 期间在美国约翰霍普金斯大学接受博士后训练，2017 年正式加入中国科学院。曾获国际人类前沿科学奖、美国马里兰干细胞研究基金奖、中国科学院院长特别奖（特等奖）、中国科学院优秀博士论文奖、青年论坛学术新人奖等。目前致力于研究神经发育、神经内分泌肿瘤、代谢调控与青春期启动等。

Origin of Neuronal Diversity in Central Nervous System

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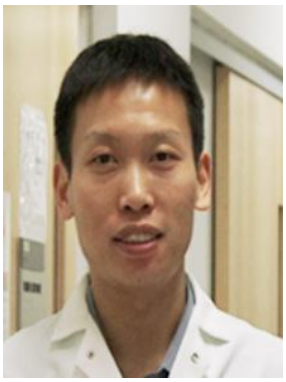
【摘要】下丘脑含有大量的神经元类型，在调控内分泌、自主神经和行为功能中发挥着举足轻重的作用。尽管过去的研究在一定程度上解析了下丘脑神经元发育的分子机制，但是其分子发育轨迹和神经元多样性的起源仍然未知。我们的研究剖析了 Rax+下丘脑神经上皮细胞衍生的 43,261 个细胞的转录组，进而绘制小鼠下丘脑的发育图谱，描绘了下丘脑的放射状胶质细胞（RGC），中间前体细胞（IPC），新生神经元和肽能神经元的发育轨迹，并通过群体谱系追踪进行验证。我们发现下丘脑 RGCs 采用保守的策略进行多能分化，但是同时能生成 Ascl1+和 Neurog2+ 两群 IPCs，而它们在端脑中则显示出完全不同的区域差异性起源。作为过渡性扩增细胞，Ascl1+ IPC 甚至可以通过命运双向性来产生谷氨酸能和 GABA 能神经元，这与端脑中的 Ascl1+ IPC 的命运非常不一样。重要的是，下丘脑的神经元分类可以被各种转录因子、神经递质和神经肽所编码，我们把这些神经元分成 29 个亚型。然后，我们通过命运调节子分析确定了它们的命运决定因子，并进一步发现处于新生状态的幼稚神经元具有分化为多种肽能神经元亚型的潜力。最后，我们进行了克隆分析以揭示单个 RGC 的增殖潜力及其产生多种神经元亚型的能力。综上所述，我们的研究提供了下丘脑发育的单细胞图谱框架，并揭示了沿谱系等级顺序的多种细胞类型以逐步递进的方式促进了下丘脑神经元的命运多样化，这表明级联多样化模型可以解释神经元的起源多样性。

【关键词】

陈家东简介



陈家东，博士，浙江大学医学院脑科学与脑医学系研究员，博士生导师，浙江大学附属第二医院双聘教授。研究神经系统发育的细胞多样性、功能以及神经系统疾病发生的分子机制。研究成果以第一作者和通讯作者发表在 Science, Cell, Cell Stem Cell, Neuron，Journal of Neuroscience, Glia 等神经科学和干细胞科学领域顶级杂志。

Multi-model Single Cell Analyses Revealed Cellular and Molecular Heterogeneity in Brain Development and Disease

陈家东
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【**Abstract**】One major obstacle in neuroscience is the cellular heterogeneity of the brain. Recent advances in single-cell genomics revealed molecular identities of diverse cell types in the developing brain. To connect the molecular and functional diversity of different cell types in the central nervous system, we established multi-model single-cell analyses integrating calcium imaging with single-cell RNA sequencing to dissect functional properties of cell subtypes in the developing human brain. In the adult brain, we integrated whole-cell patch clamp recordings with single-cell RNA sequencing to examine the molecular, morphological, and electrophysiological characteristics of dysmorphic neurons in medically intractable epilepsy. Our results revealed morphological and electrophysiological characteristics of dysmorphic neurons in focal cortical dysplasia, and imbalance of synaptic excitation and inhibition of dysmorphic neurons may contribute to seizure genesis in focal cortical dysplasia.

赵 冰 简 介

赵冰，复旦大学生命科学学院研究员、博士生导师，遗传工程国家重点实验室“类器官研究中心”主任，附属中山医院双聘教授。国家优秀青年基金获得者，国家重点研发计划干细胞专项首席科学家（青年），“东方学者”特聘教授，上海市青年拔尖人才。2013 年于清华大学取得博士学位；后于美国辛辛那提儿童医院医学中心从事博士后研究；2017 年受聘复旦大学。利用小鼠遗传、类器官模型，研究成体干细胞命运决定机制，并发展类器官培育新策略。以第一/通讯作者身份于国际权威期刊 Nature Communications、Cell Research、Protein & Cell 等发表系列研究论文，为再生生物学发展提供重要基础。代表性研究成果获 Nature News 专访报道、Nature 官网主页高亮，获国际权威综述期刊 Nature Reviews Molecular Cell Biology、Nature Reviews Gastroenterology & Hepatology 等高度评价。

感染性疾病与发育缺陷的类器官模型构建

赵冰
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【摘要】人源类器官高度模拟人体组织器官细胞类型、结构和功能，被用于构建多种发育和疾病模型。研究者构建了人源肝脏类器官新冠病毒感染模型，证实新冠病毒直接侵染呼吸系统外组织器官，并为新冠致病机制研究和药物开发提供重要模型；研究者通过在人胚肝类器官中进行基因操纵，构建了肝母细胞瘤发生的类器官模型，阐述了人肝母细胞瘤的发生机制，并提出潜在干预方案；研究者通过解析人甲状腺胚胎发育图谱和机制，构建了人胚甲状腺类器官发育模型，成功在体外诱导出可分泌甲状腺素的成熟人甲状腺类器官。上述工作为深入理解感染性疾病与发育异常导致的缺陷性疾病提供了重要理论基础、研究工具和潜在干预策略。

华国强简介

华国强，中国科学院生物物理研究所博士。2009 至 2015 年在美国纪念斯隆凯特琳肿瘤研究中心从事肠道干细胞损伤再生及肿瘤放疗研究。2015 年受聘复旦大学上海医学院组建干细胞再生生物学实验室。课题组目前研究的重点是癌症患者的个性化精准治疗和组织损伤再生。课题组依托于最新的干细胞类器官（organoid）培养技术，包括正常组织类器官和肿瘤类器官，并在此基础进行多种抗肿瘤药物和正常组织促再生新药的高通量筛选。

类器官在伴随诊断和新药研发中作用和进展

华国强
复旦大学

【摘要】类器官是由干细胞或前体细胞培养形成的，通过定向分化和谱系定型进行自组织的，具有特定器官的关键结构和功能特性的 3D 类组织培养物。类器官的兴起和发展为临床医学提供了重要的疾病模型。患者来源的类器官可以作为模拟和研究疾病模型，帮助临床医生更好地理解相关疾病病理生理，制定相应治疗策略；肿瘤患者类器官可以为药物筛选提供更准确的平台，帮助和指导临床医生为癌症患者选择最合适的治疗方案，提高患者临床预后。本会议报告将介绍患者来源类器官在精准医学和新药研发中的应用和进展。

章永春简介

章永春，上海交通大学生命科学技术学院，长聘教轨副教授，博士生导师，课题组组长，入选上海市海外高层次人才计划青年项目。2010 年于南开大学获得学士学位，2015 年于美国罗切斯特大学获博士学位，后于美国哥伦比亚大学从事博士后研究。目前课题组主要利用 3D 类器官、干细胞和转基因小鼠研究食管发育、癌症以及其他疾病的形成与治疗。本人以第一或通讯作者在国际权威期刊 Cell Stem Cell 等发表多篇论文。

干细胞与食道发育和癌症

章永春
上海交通大学

【摘要】人类多能干细胞 hPSC 包括胚胎干细胞 ESC 和诱导多能干细胞 iPSC 具有分化成所有类型细胞的全能性，为研究器官的发育、再生和疾病的形成提供了全新的途径。体外人源 3D 类器官能够很好地模拟人体内器官的结构和功能，是新兴的研究人体器官生理功能和疾病形成的重要手段。本人结合人类多能干细胞、3D 类器官以及转基因小鼠重点阐述了调控食管发育的信号通路及其机制，鉴定了巴雷特食管的形成机理以及食管癌新的治疗方法。本次报告主要细分为以下三部分类容：一、首次利用体外分化系统将人类多能干细胞高效地分化为食管上皮祖细胞；二、结合人类多能干细胞衍生的 3D 食管类器官和转基因小鼠发现调控食管发育中的新型信号通路；三、利用 3D 肿瘤类器官培养鉴定调控食管癌形成的新机制。

高 栋 简 介

高栋，博士，中国科学院分子细胞科学卓越创新中心（原中科院上海生化与细胞所）研究员。2011 年毕业于北京大学，获理学博士学位；2011 年至 2016 年先后在美国约翰霍普金斯大学和纪念斯隆凯特琳癌症研究所接受博士后训练，从事肿瘤生物学研究 2016 年 8 月加入中科院上海生化与细胞所。高栋博士开发和建立了多种组织类器官培养体系，利用多学科交叉的方法研究前列腺成体干细胞谱系命运转变，系统阐明了前列腺肿瘤细胞命运决定的分子机制等科学问题；共发表高水平论文 30 多篇，包括以通讯或共同通讯作者身份在 Nat Genet、Cell Stem Cell、J Clin Invest、Adv Sci、Nat Commun 等期刊发表的多篇论文；获得国家青年人才计划、中科院创新交叉团队、上海市青年优秀学术带头人和国家杰出青年科学基金等项目支持。

前列腺细胞谱系的命运决定

高栋
中国科学院上海生命科学研究院

【摘要】前列腺癌是欧美国家发病率第一的男性恶性肿瘤，其在中国男性的发病率也呈逐年上升趋势。但是前列腺成体干细胞的身份属性不明，发现生理状态条件下的前列腺成体干细胞是前列腺研究领域的重要科学问题。发现成为前列腺研究领域的巨大挑战。为了寻找前列腺中的成体干细胞，我们通过对成年雄鼠前列腺进行高通量单细胞转录组测序，发现了三群新的前列腺管腔细胞(Luminal-A、Luminal-B 和 Luminal-C)，分析发现 Luminal-C 具有前列腺干细胞的潜能，并特异表达 Tacstd2、Ck4 和 Psca。Luminal-C 主要分布在前列腺管腔内陷末端(Dist-Luminal-C)和近侧端的前列腺(Prox-Luminal-C)，并证实此类细胞可以高效的在体外形成前列腺类器官。利用 Luminal-C 特异的谱系示踪小鼠，我们发现 Luminal-C 是一群去势抵抗性前列腺细胞，并且证实 Luminal-C 在前列腺组织损伤修复过程中 Luminal-C 通过自我更新和分化的机制促进前列腺组织的损伤修复，明确了 Prox-Luminal-C 只参与前列腺近侧端的损伤修复。我们构建了两种小鼠自发前列腺癌模型，发现与 Luminal-A 和 Luminal-B 相比 Luminal-C 可以更高效的形成前列腺癌样增生，而且癌变的 Luminal-C 子代细胞维持了 Luminal-C 的干细胞特征，证明了 Luminal-C 具有前列腺肿瘤祖细胞潜能

徐仁和简介

徐仁和，东京大学博士，澳门大学健康科学学院特聘教授、副院长，中国干细胞研究协会和细胞治疗协会理事，澳门干细胞研究协会创始会长。长期从事干细胞研究和治疗应用，发表论文 80 余篇，被引用近 8000 次，并获得多项中美专利。最先闡明 BMP / FGF / TGFb 信号通路对人胚干细胞多能性的关键调控作用；发现细胞成球后进入“冬眠”可在常温下储运。他发明的人胚干细胞来源的 T-MSC 已授权爱姆斯坦生物科技公司使用，获美国 FDA 批准進入临床实验。

源自人胚干细胞的准细胞药：T-MSC

徐仁和
澳门大学健康科学学院

【摘要】与传统治疗不同，细胞药物可主动迁移到靶组织或靶细胞、通过多种机制发挥作用。特别是干细胞药物，已成为世界各国竞相研究的热点领域。但各国的干细胞药物绝大多数仍处于研究阶段，真正上市的干细胞药物很少。间充质干细胞（MSC）通过旁分泌、细胞分化和免疫调节用于多种疾病包括新冠肺炎的临床试验治疗。但大多数临床试验止于早期，其原因主要包括 MSC 质量波动；长期安全性未能确立；细胞产量跟不上。我们发现人多能干细胞可经胚外的滋养层细胞分化为 MSC（简称为 T-MSC）。T-MSC 具有以下优点：（1）人多能干细胞是稳定、不竭的来源；（2）产量、质量和安全性可控；（3）方便基因操作；（4）MSC 本身有免疫调节作用且无需长期移植。T-MSC 用于小鼠和猴子多发性硬化、炎性肠病、自发性骨关节炎以及皮肤损伤模型的治疗取得显著疗效，获得中美专利和美国 FDA 批准进入多发性硬化的临床试验。另外，我们还发现干细胞成球后进入“冬眠”可在常温下储运，大大方便了干细胞的远程治疗。

章 梅 简 介

章梅，中国科学技术大学副研究员；长期从事于人视网膜发育与衰老机制研究，基因编辑与干细胞相关的视觉修复等研究工作。2011 年获香港大学博士学位，2011-2016 年在约翰霍普金斯金斯大学从事博士后研究。相关文章发表在 Science Advances ， National Science Review, Science Bulletin, Developmental Cell 等国际期刊上。

视网膜类器官表观遗传修饰与其命运决定

章梅
中国科学技术大学

【摘要】人视网膜是由多种类型的神经元和神经胶质细胞组成的复杂而精细的神经感觉组织。体外由人多功能干细胞分化而来的人视网膜类器官被广泛应用于模拟人胚胎视网膜发育进程，视网膜疾病生成与药物研究。然而，有关人胚胎视网膜和视网膜类器官在发育进程上的表观遗传调控和基因表达的差异尚不清楚。通过对不同发育阶段的人胚胎视网膜和视网膜类器官进行多组学分析，我们解析其在发育过程中的染色质可及性图谱和相关的基因表达水平变化，并通过染色质开放特征将人胚胎视网膜和类器官的发育阶段相似程度进行匹配，从而分析它们在染色质调控和基因表达特征上的共性与差异。研究发现人胚胎视网膜和类器官有着类似的重大发育转换阶段，并与染色质开放变动精准对应。研究重构了人胚胎视网膜和类器官发育过程的转录因子调控网络，发现了新的参与人视网膜发育过程的转录因子，并利用视网膜类器官进行了相应的功能验证。系统而全面地建立了“人视网膜分子机制发现–视网膜类器官基因操作验证”的闭环研究体系，解决了当前难以直接在人视网膜上进行基因操作和功能验证的难题。

王 树 简 介

王树，中国科学院化学研究所研究员。1994 于河北大学获学士学位， 1999 于北京大学获博士学位，1999 - 2001 中国科学院化学研究所博士后，2001 - 2004 美国加利福尼亚大学圣巴巴拉分校博士后。曾获国家杰出青年基金、第十二届中国青年科技奖、科技部创新领军人才、北京市科学技术一等奖。主要研究方向：生物功能材料设计与合成、功能有机共轭分子体系的设计与生物传感应用、生物自组装体系构筑及其抗菌与抗肿瘤活性研究、生物电子以及化学生物学。在 J. Am. Chem. Soc.、 Angew. Chem. Int. Ed.、 Adv. Mater.、 Chem. Rev.、 Acc. Chem. Res.、 Chem. Soc. Rev.、 Sci. Adv.、 Nat. Commun.、 Nat. Protocol.等期刊发表学术论文 280 余篇，申请/授权专利 40 余项。目前担任美国化学会 ACS Applied Bio Materials 期刊 Deputy Editor。

功能高分子生物材料设计与合成

王树
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【摘要】开发具有优性能的的 3D 打印功能墨水，实现干细胞活性维持及分化，具有重要的意义。我们设计了一种新型阳离子聚噻吩材料 PMNT，并将其整合到明胶/海藻酸盐基质中，获得了新型 3D 生物打印墨水。在无血清培养基中，PMNT 通过驱动细胞周期和上调生物合成代谢途径中的特异性基因表达，显著克服了缺乏营养的环境，有效促进了人脐带间充质干细胞（hMSC）的增殖，并将生物打印技术与 hMSCs 结合，加快了大鼠全层切除创面的愈合。为生物打印结合干细胞用于再生组织治疗提供了新途径。我们设计合成了聚氨酯与明胶的复合材料，利用两种材料各自的优势开发了新型生物 3D 打印复合墨水，通过共价交联方式形成弹性材料。复合墨水能满足挤出式打印的要求，显著提高材料固化后的拉伸弹性和剪切模量。有潜力成为一种用于组织和器官生物打印的弹性材料。 另外，我们通过提高胶原蛋白的溶解度并改善挤出打印性能制备了胶原蛋白墨水，光谱结果表明胶原蛋白保留了三股螺旋结构。利用化学方法在胶原蛋白侧链修饰可交联基团，通过光固化提高打印后结构的稳定性。细胞打印结果表明细胞存活率高，为生物打印方法构建多分支血管网络提供了新材料。

黄卫华简介

黄卫华，武汉大学化学与分子科学学院教授，博士生导师，国家杰出青年科学基金获得者，国家“万人计划”科技创新领军人才。分别于 1996 与 2002 年在武汉大学获得学士和博士学位，然后留校任教。2005 年 3 月到 2006 年 3 月在法国巴黎高师(ENS)从事博士后研究，2007 年 11 月晋升为教授。从事生命分析化学研究，主要研究方向为单细胞分析、生物电分析化学以及微流控芯片。

细胞生命活动高时空分辨电化学监测

黄卫华
武汉大学化学与分子科学学院

【摘要】细胞是生命体结构和功能的基本单元，快速准确获取细胞生化信息有助于我们深入理解生命过程。微纳米电化学传感器具有灵敏度高、响应迅速、空间分辨率高等特点，在单细胞与亚细胞实时动态监测方面具有独特优势。最近，我们提出一种基于核壳结构纳米线构建纳米电极的新策略，进一步利用物理吸附、静电组装以及共价键合等原理，实现了多种纳米材料和生物酶在纳米电极表面的可控功能化，在此基础上实现了胞内重要活性分子（活性氧、NADH 和葡萄糖）的实时动态监测。此外，我们对巨噬细胞吞噬溶酶体 ROS/RNS 稳态调控机制、神经元囊泡内谷氨酸胞吐释放动力学过程进行了探究，在单/亚细胞水平提升了对这些生命过程的认知。另一方面，针对传统硬质电化学传感器难以顺应细胞及组织形变的挑战，我们制备了高长径比且电化学性能优异的金纳米管和碳纳米管材料，构建了柔性可拉伸电化学传感器[7]，进一步利用复合纳米结构提升了传感器检测灵敏度、选择性、生物相容性以及光致可更新性能。在此基础上实时动态监测了内皮细胞、软骨细胞以及肠道组织在力学刺激下的信号分子动态释放，充分展示了柔性可拉伸电化学传感策略在细胞及组织力学信号转导研究方面的独特优势及前景。

张 旭 简 介

张旭，中科院大连化物所副研究员。中科院大连化物所博士毕业，美国杜克大学博士后，于 2020 年回国。主要从事血管与肌肉器官芯片病生理模型构建与药物评价研究，为心血管疾病体外模拟提供新策略。在 Nature Communications、Advanced Materials、Small、Advanced Science 等国际重要刊物发表论文 20 余篇，授权国内发明专利 12 项，参与撰写英文书籍一本。

功能性血管/肌肉组织芯片构建及应用

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【摘要】血管和肌肉是人体全身性组织，是循环系统与运动系统的重要组成部分，和能量代谢、免疫调节、衰老等人体重要机能戚戚相关。血管和肌肉组织的病变会引起冠心病、脑卒中、能量代谢失调、运动功能障碍等一系列复杂性疾病。本报告以器官芯片技术为核心，将组织工程、细胞生物学、生物材料等多学科交叉融合，在体外构筑结构与功能兼具的人体血管、肌肉微生理系统。并系统地展开动脉粥样硬化模型构筑和肌肉疾病体外模拟研究与药物评价应用，为相关疾病的体外建模、机制解析、新药开发提供了新策略。

蒋田仔简介

蒋田仔，研究员，中国科学院自化研究所脑网络组研究中心主任，脑网络组北京市重点实验室主任。欧洲科学院外籍院士(MAE)，IEEE Fellow, IAPR Fellow，AIMBE Fellow。现任 Neural Networks, IEEE Transactions on Cognitive and Developmental Systems 等多种国际刊物编委，北京脑网络组与类脑智能学会理事长，中国神经科学学会常务理事和意识与意识障碍分会会长。获国际脑电图与临床神经科学学会的最高成就奖(Turan Itil Award，2021)，国际神经网络学会终身贡献奖 (Hermann von Helmholtz Award，2020)，吴文俊人工智能杰出贡献奖（2020），北京市自然科学奖一等奖（2021），国家自然科学奖二等奖(2004)等。

多模态跨尺度灵长类脑网络组图谱绘制方法和应用

蒋田仔
中国科学院自动化研究所

【摘要】本报告介绍脑网络组和脑网络组图谱的研究背景和研究内容，包括脑网络组的定义，脑网络组与脑连接组的区别，脑网络组的主要研究方向，脑网络组图谱绘制的思想以及与现有脑图谱发的本质区别等方面以及灵长类脑网络组图谱跨物种比较研究；最后对未来研究方向进行总结和展望。

柳夏林简介

柳夏林，教授/主任医师，博导，中山大学中山眼科中心医务处处长。长期从事致盲眼病的发病机制及干预研究，提出临床转化研究新策略。在眼新生血管病变、眼免疫炎症机制以及干细胞再生医学研究方面进行了系列研究并取得了重要工作成果。柳夏林团队目前在国际有影响力的期刊已发表SCI论文80多篇，包括PNAS, PLoS Med, Sci Adv., Nat Commun., Cell Death Diff, IOVS.等。近年负责承担的项目包括：国家重点研发计划项目（负责人）、国家自然科学基金重点项目（负责人）、广东省自然科学基金重点项目（负责人）、广州市科技计划项目（负责人）等。

干细胞外泌体用于干眼治疗的作用机制研究

柳夏林
中山大学中山眼科中心

【 **Abstract** 】 Graft-versus-host disease (GVHD)-associated dry eye disease causes unbearable pain and significant visual impairment. Current drugs provide limited benefits and many patients are refractory to therapy. It is the impetus to develop effective therapies or biologics for this devastating disease. Here we report that exosomes from mesenchymal stromal cell (MSC-exo) administered as eye drops significantly alleviates GVHD-associated dry eye by suppressing inflammation and preserving corneal epithelium in mouse models and humans. We show here that twenty-eight eyes with refractory GVHD-dry eye disease that were enrolled in a clinical trial (NCT04213248) presented significant relief and diminution of dry eye symptoms after MSC-exo treatment. MSC-exo therapy promoted recovery from dry eye and maintenance of ocular surface homeostasis by reprogramming pro-inflammatory M1 macrophages towards the immune-suppressive M2 phenotype. Taken together, MSC-exo eye drops are efficacious in treating GVHD-associated dry eye.

郑雄飞简介

郑雄飞，博士，研究员。浙江衢州人，本硕博毕业于哈尔滨工业大学，博士课题为可降解骨支架生物 3D 打印研究。现任中国科学院沈阳自动化研究所/机器人学国家重点实验室/器官制造课题组长，辽宁省“兴辽计划”青年拔尖人才，科技部“智能机器人”重点研发计划项目首席科学家，中科院"器官制造与重建”先导 A 专项课题负责人。从 2004 年起开展生物制造研究，多年来坚持科学与技术互动的研究策略，已带领团队建立起工程基础扎实、多学科充分融合交叉的器官制造技术体系及初步的理论框架。 近几年，重点攻关血管化大尺度肝脏制造，学术成果发表在 Biofabrication，Advanced Healthcare Materials 等杂志上，并获 ACS AMI 期刊封面报道。 在科技部项目支持下，与第四军医大学合作完成了国际首例 3D 打印可降解椎间融合器的临床转化，相关技术目前已在四军医、北京协和医院临床应用 60 余例，安全性和有效性得到初步验证。个人及团队的理想是“器官制造，造福临床”。团队的文化是“目标清、可考核、用的上、有影响”。

复杂器官制造装备与工艺探索

郑雄飞
中国科学院沈阳自动化研究所

【摘要】再生医学的发展，已经实现了简单组织如骨及皮肤的修复重建。但是对于像肝脏这种复杂器官的构建，当前干细胞及组织工程手段仍面临重大挑战，通过生命科学与工程制造技术的结合，开展复杂器官的直接制造被认为是一条可行的技术途径。

本报告将分享领域内复杂器官构建的最新进展，汇报中科院沈阳自动化所器官制造团队在该方向上的突破和思考：包括复杂器官设计方法、器官制造装备、器官制造工艺及应用案例。

阴正勤简介

阴正勤，陆军军医大学第一附属医院眼科，主任医师、博士生导师，第十一届中华医学会眼科学分会副主任委员、第九届全军眼科学会主任委员、第三届和第六届重庆市医学会眼科专委会主任委员，国家干细胞项目 973 首席科学家、国家杰出青年科学基金获得者。军队技术二级教授、澳大利亚新南威尔士大学客座教授、何氏眼科集团医疗首席科学家。获亚太眼科学会防盲杰出贡献奖、中华眼科杰出贡献奖、中美眼科学会金钥匙奖。在干细胞治疗视网膜变性疾病方面处于国际先进水平。

hEROs-C-Kit+/SSEA4-RPCs 移植治疗视网膜变性疾病的有效性机制研究

阴正勤
陆军军医大学第一附属医院（西南医院）

【摘要】胚胎干细胞（ESCs）具有多能性，在体外可大量扩增，且相较于 iPSCs，具有更好经济效益比，获取周期短等特点，但成瘤风险高限制了 ESCs 来源细胞的临床转化。成体干细胞（ASCs），安全性较好，成瘤风险低，但细胞来源困难、难以标准化和产业化。如何将 ESCs 和 ASCs 的优势结合起来，得到来源容易、能标准化和产业化、成瘤风险低、分化潜能高、有效性能够持续的理想的治疗细胞来源，是干细胞临床转化的关键科学问题。ESCs 来源的视网膜类器官（hEROs）在体外可大量、稳定地获得，降低了成瘤风险。hEROs-RPE 与自然分化法诱导的 hESC-RPE 细胞相比，具有更多视网膜色素上皮前体的生物学特性。其细胞形态、超微结构、极性和全基因组表达谱更接近于成体 hfRPE 细胞。移植 RCS 鼠视网膜下腔后能够保护视网膜感光细胞和挽救视功能。hEROs 可能包含有未分化完全的早期胚胎细胞，对筛选神经视网膜来源的祖细胞（RPC）增加了成瘤风险。本研究首次采用细胞表面标记（C-Kit+/SSEA4-）联合筛选从 hEROs 分离出 C-Kit+/SSEA4-视网膜祖细胞（RPCs），并证实其与 hfRPCs 基因表达模式相似，是具有自我更新能力、多向分化潜能及较低的成瘤风险的 RPCs 亚群。C-Kit+/SSEA4- RPCs 移植治疗视网膜变性鼠安全有效，其治疗有效性可能是通过细胞替代及物质交换和改善视网膜变性微环境实现的。以上研究表明 hEROs-RPE 和 hEROs 来源 C-Kit+/SSEA4-RPCs 兼具成体干细胞和胚胎干细胞优势，是临床转化理想的治疗细胞。

欧阳宏简介

欧阳宏，中山大学中山眼科中心研究员，博士毕业于中国农业大学，并先后进入美国哈佛大学医学院细胞生物学系及加州大学圣地亚哥分校眼科中心做博士后研究。主要致力于干细胞稳态与眼部疾病机理及干细胞治疗致盲性眼病的临床转化研究。承担国重研发计划干细胞及转化研究青年项目，国自然优秀青年基金、国际合作等项目。

自体干细胞与角膜盲疾病治疗

欧阳宏
中山大学中山眼科中心

【摘要】角膜位于眼球最外层，不仅是保护眼内组织的结构屏障，也是眼部重要的屈光介质。位于角膜边缘处的角膜缘干细胞（Limbal Stem-cell, LSC）是角膜上皮自我更新的主要来源，在角膜上皮损伤修复及稳态维持方面发挥至关重要的作用。严重的角膜疾病常常导致 LSC 丧失或衰竭（limbal stem-cell deficiency，LSCD），进而引起角膜结膜化、新生血管侵入等病变，并最终致盲，是世界上主要的致盲眼病。对于这类疾病，补充 LSC 是唯一有效的治疗方式。然而，LSC 增殖与分化机制不清，移植细胞存活率低等问题制约了临床转化应用。在 LSC 命运调控机理研究中，我们描绘了人类 LSC 的组蛋白修饰和染色质可及性图谱，构建了基于超级增强子与转录因子互作的转录调控网络。发现 RUNX1 和 SMAD3 是 LSC 的命运决定和稳态维持必不可少的转录因子，与眼发育重要因子 PAX6 相互作用，形成特异的核心转录环路。并进一步揭示，破坏 RPS 的协同作用与多种常见角膜盲疾病相关。在 LSC 再生角膜转化研究中，我们创建了无血清、无滋养层 LSC 扩增培养体系，并利用角膜基质干细胞联合移植，促进 LSC 的定植与角膜重建。

李 炜 简 介

李炜，厦门大学眼科研究所教授，博士生导师。现任厦门大学医学院副院长，眼视光学系主任；历任中华医学会眼科学分会基础研究发展委员会委员、角膜病学组委员，中国医师协会眼科分会角膜病学组委员，海峡两岸医药卫生交流协会眼科专委会常委、总干事，中国老年保健协会眼科专委会委员，福建省医学会眼科分会角膜病学组组长，福建省医师协会眼科医师分会副主委，亚洲干眼协会委员；中华眼科杂志、中华实验眼科杂志、中华眼视光杂志通讯编委；Exp Eye Res 等国际杂志编委。李炜教授主要从事角膜上皮干细胞、角膜组织工程、干眼的基础与临床研究，先后承担 15 项国家及省部级科研项目。发表 SCI 论文 90 余篇，获得 17 项美国专利及 10 项中国专利，参编 7 部专著或教材。获得福建省科技进步一等奖（3 项）、厦门市科技进步一等奖（3 项）、运盛青年科技奖、中华医学科技奖、紫金科技创新奖、中华眼科学会奖、福建医学科技奖等奖项；获得福建省新世纪优秀人才、福建省科技创新领军人才、福建省杰出青年基金获得者、厦门市“双百计划”海外高层次人才、领军型创业人才等荣誉称号。

角膜上皮干细胞治疗的现状和挑战

李炜
厦门大学眼科研究所

【摘要】以角膜上皮干细胞缺乏为特征的严重眼表面疾病仍是眼科临床面临的重要致盲性眼病。角膜上皮干细胞部分缺乏可以通过羊膜移植或自体健眼角膜上皮干细胞移植获得成功治疗。严重的角膜上皮干细胞缺乏可以采用阶段性自体角膜上皮干细胞移植、异体角膜缘移植、体外构建的组织工程角膜上皮移植等方法治疗。随着干细胞与组织工程技术的发展，组织工程角膜上皮移植技术有可能在临床得到大规模应用，但该技术目前仍面临技术标准化等方面的挑战。随着对干细胞微环境认识的不断深入，基于微环境的角膜上皮干细胞缺乏治疗技术也是未来发展的重要方向。

池在龙简介

池在龙，温州医科大学研究员，省特聘教授，眼科学与视觉科学国家重点实验室 PI，附属眼视光医院遗传眼病专科主任。日美留学十多年，一直从事于视神经损伤修复、眼底新生血管以及炎症免疫相关性眼病等主要不可逆致盲性眼病的基础与应用研究，在眼病遗传以及新药研发等领域发表连续性论文。近五年课题组主持国家级和省部级项目 10 余项。基于外泌体的眼病诊疗以及新药研发等研究成果，申请国家发明专利 11 项，已授权 5 项。

不同干细胞源外泌体在眼病治疗中的应用

池在龙
温州医科大学附属眼视光医院

【摘要】外泌体是一类可由所有活细胞分泌的细胞外囊泡，可携带细胞内生物活性分子，免疫原性低、易通过血脑屏障，因此被认为是无细胞的细胞替代疗法。同时，外泌体作为天然的纳米级细胞外囊泡，其磷脂双层膜结构可保护 RNA 等生物分子不易被降解，并可避免机体的免疫清除，在药物递送方面独具优势。已有研究表明，多能诱导干细胞（iPSC）、间充质干细胞（MSC）来源的外泌体在脑和视网膜神经节细胞（RGC）损伤中均显示神经保护作用，神经祖细胞（NPC）以及小胶质细胞来源的外泌体可减轻光感受器细胞凋亡等。鉴于此，我们分别收集人 iPSCs 及其定向分化 NPC 分泌的外泌体，探讨对视神经损伤模型 RGC 保护作用。同时，利用雪旺细胞分泌的外泌体进行模型动物表型研究。结果显示，各种来源外泌体均显示不同程度的神经保护作用，也印证了外泌体的微环境调控作用。进一步，原代 RGC 共培养实验体系中阻断雪旺细胞外泌体的分泌，可逆转其促进神经轴突生长作用。外泌体具备良好的临床应用前景，或可为临床提供崭新的治疗策略。

刘 君 简 介

刘君，北京大学生命科学学院、北大清华生命科学联合中心研究员。实验室主要致力于研究 RNA 表观遗传修饰/non-coding RNA 的分子调控机制和相关生物功能。RNA 表观遗传修饰在真核生物的很多生理过程中起到了重要的调控作用，它和发育、癌症、抗肿瘤免疫的密切相关性也暗示了基础研究和转化医学结合的可能性。实验室一方面会结合表观遗传学、分子生物学、细胞生物学、基因组学等研究手段，全面揭示 RNA 表观遗传修饰/non-coding RNA 对于染色质结构和基因转录的分子调控机制，另一方面也会探索其重要的生理、病理学意义，以期在相关疾病中发现有效分子靶标并开发新型疗法。主要研究方向：（1）RNA 表观遗传修饰对于染色质结构和基因转录的分子调控机制。（2）细胞核内 non-coding RNA 稳定性的分子调控机制。（3）RNA 表观遗传修饰/non-coding RNA 对于生物大分子相变过程中的调控。

The Functional Roles of RNA Modification in Regulating Transcription and Chromatin State

刘君
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【**Abstract**】Over 150 types of post-transcriptional RNA modifications have been identified. Among them, N^6-methyladenosine (m^6A) is the most abundant mRNA modification in eukaryotes, it has been known to affect almost every stage of mRNA processing, including splicing, export, translation and decay. The regulatory roles of m^6A on RNA metabolism have been well defined in various biological processes. Here I will discuss a new mechanism about how m^6A regulate gene transcription and chromatin state. m^6A has been reported to play critical roles in self-renewal and differentiation of embryonic stem cells through YTHDF2-mediated cytoplasmic decay. m^6A also appears to exhibit YTHDF2-independent regulation during early development, given that Ythdf2 knockout mice can survive to late embryonic developmental stages, while Mettl3 knockout results in early embryonic lethality. We found that knockout Mettl3 or a nuclear reader Ythdc1 induced chromatin openness and transcription activation in an m^6A-dependent manner in mouse embryonic stem cells (mESCs). We confirmed that METTL3 deposits m^6A modifications on chromosome-associated regulatory RNAs (carRNAs). And the m^6A modification of carRNAs induced their degradation through YTHDC1 mediated NEXT nuclear degradation pathway and thus decreased downstream gene transcription. We applied dcas13b-FTO system to site-specific

demethylation on carRNAs and observed elevated transcript levels, opened local chromatin state, and activated downstream transcription. We further linked the changes of the m^{6}A-mediated carRNAs to mESCs differentiation and renewal. Notably, my further studies showed that downregulation of METTL3 also stabilized carRNAs in endometrial cancer cell line, together with a more open chromatin and faster cell migration. Altogether, this work has demonstrated a new layer of transcription regulation by m^{6}A-modified carRNAs which is distinct from mRNA methylation effects, and has enlightened that more roles of ncRNA m^{6}A methylation remain to be revealed in transcription control during various biological processes.

吕赫喆简介

吕赫喆，中国科学院动物研究所研究员。长期以细胞及小鼠等为研究模型，研究细胞异质性与命运决定机制。绘制乳腺癌单细胞基因图谱，鉴定调控细胞异质性核心基因，探寻肿瘤干细胞命运轨迹，发现异质性发生的全新机制；揭示肿瘤异质性导致的肿瘤耐药机制，发现隐藏于肿瘤组织中的关键细胞具备 PAK 信号的高度激活，证明 PAK 抑制剂可以改变耐药肿瘤细胞命运，诱导细胞死亡。研究论文发表在 Nature，Dev Cell，Cell Reports 等国际学术期刊。

细胞异质性与肿瘤干细胞

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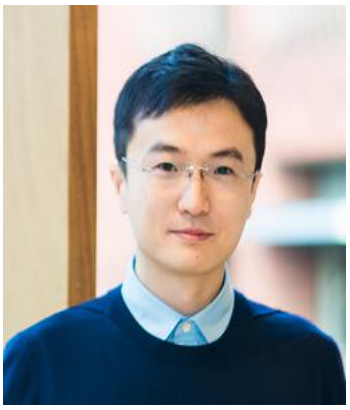
【摘要】肿瘤异质性是肿瘤转移和耐药的关键，是肿瘤难以治愈的根本原因。肿瘤干细胞被认为是肿瘤异质性产生的主要因素之一，然而对肿瘤干细胞本身的认知却仍不完整。肿瘤干细胞向肿瘤细胞分化的过程也不是单向的，已分化的肿瘤细胞仍然可以去分化重新获得干细胞的特征。我们以肿瘤为模型，研究肿瘤异质性的发生机制，以及肿瘤异质性调控的转移、耐药机理，解析了 MAPK 和 PAK 等重要信号通路在细胞异质性发生和疾病演变过程中的调控网络，追踪不同肿瘤单细胞在发生新基因突变和药物处理后的命运轨迹，绘制肿瘤单细胞基因图谱，探寻肿瘤异质性发生机理，为新药开发和创新疗法提供理论基础和实验依据。

张 兵 简 介



张兵，博士　　2007 年毕业于山东大学，2015 年获得美国凯斯西储大学生物化学博士学位；2015-2020 年在美国哈佛大学干细胞与可再生生物学系任职博士后，从事哺乳动物皮肤干细胞调控与皮肤和毛发再生的研究。曾先后获得哈佛大学杰出教学奖和 Charles A. King Trust 博士后奖金。2020 年秋季入职西湖大学任研究员，研究方向为皮肤干细胞的调控以及皮肤和毛发再生。

Stem Cell-niche Interactions in Regeneration and Stress

张兵
西湖大学

【**Abstract**】Psychological stress negatively affects tissue homeostasis and regeneration, but whether and how stress perception leads to profound changes in tissue biology remains poorly understood. Here, we investigate this question in the skin. Psychological stress has been anecdotally associated with hair graying, but a scientific evidence linking the two is lacking. By adapting approaches to induce stress in mice, including physical pain and restraining, we showed that psychological stress leads to gray hair formation through rapid depletion of melanocyte stem cells (MeSCs). Combining denervation, endocrine surgeries, cell ablation, and cell-type specific gene deletions, we showed that stress-induced hair graying is independent of stress hormones or the immune system, but relies on the activation of the sympathetic nervous system. Sympathetic nerve terminals innervate the MeSC niche. Under stress, sympathetic nerve activation leads to burst release of neurotransmitter norepinephrine, which targets MeSCs directly. Norepinephrine drives MeSCs proliferation, leading to their rapid exhaustion. Inhibition of MeSC proliferation or MeSC-specific deletion of norepinephrine receptors rescue stress-induced hair graying. Our study shows that psychological stress-induced neural activity can alter somatic stem cells directly, and identifies strategies that might be exploited for therapeutic purposes in the future.

张 满 简 介

张满，2007 年毕业于四川大学, 2013 年获中国科学院上海生化与细胞研究所发育生物学博士学位；2014 年 7 月于爱丁堡大学从事博士后研究；2019 年 12 月起任生物岛实验室 CCLA 中心研究员。目前共发表 SCI 论文 14 篇，以第一或共同第一作者身份在 Nature, Cell Research 等权威杂志上发表论文 5 篇。入选国家海外高层次人才计划青年项目，广州市黄埔区“优秀人才”计划，主持国自然面上基金一项。

胚胎早期细胞命运转变的研究

张满
生物岛实验室

【**Abstract**】Embryonic stem cells (ESCs) can differentiate into all cell types of the embryo proper but rarely generate totipotent 2C-like cells and the trophoblast because of the epigenetic barriers. However, how the epigenetic obstacles in ESCs are established is not fully understood. Here, we show that mouse ESCs treated with HDAC inhibitors, greatly increase 2C-like (MERVL::GFP positive) cells population and can transdifferentiate ESCs into trophoblast stem like cells (TSLCs). Interestingly, lineage tracing and single-cell RNA seq show that HDACIs mediated ESCs to TSCs transition is not through the 2C-like state. Mechanismly, HDACIs inhibit Class I histone deacetylases activities in the repressor complex and increase acylation levels in the regulatory regions of both 2C and TSC specific genes to derepress these genes expression. In summary, our results provide an insight into how epigenetic changes manipulate early embryonic cell fate transition between ESCs, TSCs and 2C-like cells.

马 帅 简 介

马帅，博士。中国科学院干细胞与再生医学创新研究院“致一”研究员，干细胞与再生医学科学数据中心办公室副主任，中国科学院动物研究所副研究员。主要研究方向为衰老与再生的系统生物学，利用单细胞测序技术开展衰老、衰老相关疾病以及衰老干预新型分子机制的挖掘与解析。近年来共发表论文 16 篇，其中以第一/共同第一作者在 Cell、Cell Research 、 Nature Cell Biology 、 PNAS 、 Nucleic Acids Research（×2）、Protein & Cell 等杂志上发表论文 8 篇，文章总影响因子 260+。目前作为研究骨干参与科技部重点研发计划 1 项，主持国家青年基金项目 1 项，申请专利 1 项。代表性成果获 2020 年度“中国科学十大进展”及“中国生命科学十大进展”。获 2020 年度“中国科学院杰出科技成就奖（集体奖）”。现任中国老年医学学会基础与转化医学分会委员、中国老年学和老年医学学会抗衰老分会委员。

单细胞图谱揭示异体共生对衰老的恢复效应

马帅
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【**Abstract**】 Aging is manifested by multisystem deterioration across body, leading to pleiotropic changes in many aspects of mammalian physiology that cause declined regenerative potential, tissue failure and emergence of chronic diseases. Several ‘rejuvenating’ interventions have been documented to antagonist ageing and aging-related systemic decline, including heterochronic parabiosis, partially reprogramming, senescent cell ablation, calorie restriction, physical exercise, and pharmaceutical administration. Among them, heterochronic parabiosis in which circulation system of young and old animals were connected offer a unique experimental model to study how organism as a whole is rejuvenated by circulatory youthful factors, and vice versa is affected by blood-born circulatory aging factors, which may lead to a holistic understanding of aging and its rejuvenation.

Both process of aging and heterochronic parabiosis-mediated aging rejuvenation are strongly associated with changes in circulatory systems that are functionally integrated with immune system. Immune and blood cells are generated by hematopoietic stem cell (HSC), a multipotent cell type at the top of the differentiation hierarchy of multilineage hematopoiesis. Adult hematopoiesis in mammals happens primarily in the bone marrow (BM), including a heterogeneous mixture of blood cells at different stages of differentiation. Bone marrow give rise to peripheral blood (PB) that circulates across body, including spleen, an important

immune organ that storing and releasing certain types of immune cells that mediate tissue inflammation. HSC, BM, PB and spleen constituted immune system orchestrate local immune cells and affects homeostasis of peripheral tissue, such as skin. Age-associated immune alteration, called immunosenescence, associated with inflammageing and compromised immune efficacy, is an integral reflection of the ageing process and a driver of age- related disease. Crucially, heterochronic parabiosis is reported to repress immunosenescence and alleviate systemic aging including that of skin. However, how immunosenescence and systemic aging were counteracted by heterochronic parabiosis is still enigmatic.

It is worth noting that mammalian organisms, especially the immune system, are highly heterogeneous, composed of different cell types that responses differentially to aging and heterochronic parabiosis. Recently, we and others have reported the whole-body or multi-tissue single-cell transcriptomics of aging, which reveals massive cellular heterogeneity of aging, and proved to be an important methodology to study systemic aging. Application of this atlas to heterochronic parabiosis will certainly help us understanding how exposure to young blood affect hematopoiesis, immunosurveillance, peripheral tissue homeostasis, and thereby rejuvenate aging at whole-organism level.

Here, we systematically analyzed more than 70,000 single cells across immune system (enriched HSC, BM, PB and spleen), as well as a representative tissue skin, sampled from heterochronic parabiotic animals as well as control groups of age-matched pairs, namely isochronic parabiotic animals. Then, the data reveal how young circulatory milieu rejuvenates old animal and how old circulatory milieu compromise young animal in terms of cell type distribution, gene expression signature, and cell-cell communication networks. In summary, we constituted the first multitissue single-cell transcriptomic atlas to study the effect of heterochronic parabiosis on aging, informing both our understanding of immunosenescence and inflammaging, and the development of novel therapies that alleviate systemic aging.

曾 文 简 介

曾文，教授，教研室主任。国家重点研发计划青年科学家项目首席，国家优青，重庆市杰青获得者。中国科协青年人才托举工程第二届典型代表人物。获批陆军科技英才，担任重庆市青年专家工作室领衔专家围绕组织工程血管与干细胞开展基础和转化研究。发表 SCI 论文 20 余篇（10 分以上 8 篇）；多次在国际会议上做论坛主席或邀请报告，共同主编 SCI 专刊 2 部。作为第二完成人制定生物人工血管等产品注册标准 4 项，授权专利 3 项。

干细胞与血管再生

曾文
陆军军医大学

【摘要】干细胞结合生物材料在组织工程中的应用是干细胞治疗重要的应用场景。工程化血管在心血管疾病治疗中有重要需求，血管再生也是复杂器官再生的重要基础。内皮细胞是工程血管必不可少的种子细胞，内皮化直接影响了工程血管的通畅和功能维持。干细胞来源的内皮细胞作为工程血管种子细胞新的来源备受关注。然而 iPSC 诱导分化的内皮细胞仍然存在不稳定、效率低等问题。同时 iPSC-EC 的异质性也导致种子细胞对生物材料的响应性和对血流刺激的顺应性都存在差异，而这种差异提示可能存在适合小口径工程动脉的最优 iPSC-EC 亚群。基于此，课题组通过单细胞转录组测序解析成人不同部位小口径血管内皮特征。

在对成人冠状动脉、颈总动脉、肱动脉、股动脉、大隐静脉等血管内皮进行测序后，我们分别获得了动脉与静脉的标志性基因，并对不同部位血管内皮细胞的亚群进行了聚类分析，发现胸主动脉 AO 表现出最强的动脉内皮特征，且表现出强抗凝血特征；而冠状动脉 CA 促平滑肌生长的特征最强，动脉细胞在细胞外基质表达相关基因上富集程度明显高于静脉内皮。随后通过 iPSC 定向分化为动脉内皮细胞进行单细胞转录组测序，以人类内皮细胞转录组特征为参考精准获取最适合小口径动脉血流动力学和工程材料的种子细胞。并在成功在体外生物反应器中构建内皮化长段小口径血管，使得内皮覆盖率提升到 90%-100%。工程血管移植后，发现不同亚群种子细胞对血管

的通畅率和血栓形成有显著影响。这为进一步获取最优亚群 iPSC-EC 种子细胞构建小口径功能动脉奠定基础，也为干细胞与生物材料结合用于血管移植提供了支撑。同时，通过结合干细胞与 3D 打印技术，干细胞诱导成为肾脏内皮细胞与足细胞，种植在打印形成的复杂血管模型上，促进血管网络再生与重建，实现了肾小球仿生化微尺度血管结构单元构建。

黄 姗 简 介

黄姗，中山大学中山眼科中心，副研究员。研究方向为晶状体再生、白内障防治的基础研究。鉴定了成体晶状体存在干细胞，建立了类晶状体再生体系，研究了内源干细胞进行晶状体原位再生的分子机制（Nature，2016）。kj 探讨如何维持晶状体上皮特性，避免其发生 EMT 造成组织异常再生（Cell Proliferat，2020）。研究发表在 Nature，Cell Proliferat，IOVS，Life Sci 等期刊

HDAC6 Inhibits Fibrotic Cataract through Increasing α-tubulin Acetylation during Lens Repair

黄姗
中山大学中山眼科中心

【**Abstract**】Cataracts are the leading cause of blindness in the world. The visual axis, defined as the normal passage of light into the eye, may undergo visual axis opacification (VAO) owing to the postoperative disorganized fibrosis of remaining lens epithelial cells (LECs), leading to vision loss. In pediatric cataract patients, particularly, the incidence of the fibrotic secondary cataract is almost 100%, which could disrupt their visual development and may result in irreversible blindness. We previously identified LECs with self-renewal and differentiation ability in mammals and regenerated lenses with visual function in situ by preserving endogenous LECs and lens capsule integrity via a small wound opening surgery. Preventing trans-differentiation of LECs to mesenchymal cells and maintaining its epithelial features and regenerative potential is critical for the function repair of lens. Microtubules, the major component of the cytoskeleton, are involved in regulation of multiple biological cell processes and maintenance of cell shape. We found that LECs transdifferentiated into mesenchymal cells with decreasing of the acetylation of α-tubulin, as well as the increasing expression and activation of histone deacetylase 6 (HDAC6) during lens fibrosis after the surgery. Meanwhile, we found that the process of microtubule cytoskeleton organization, trigged by the acetylation of α-tubulin, was critical for LFCs differentiation and that the expression and activity of HDAC6, which could deacetylate α-tubulin, was decreased while α-tubulin acetylation increased during the normal differentiation of LFCs. Further, we found that α-tubulin acetylation was enhanced via HDAC6 inhibition, and thereby maintained the epithelial characteristics of LECs, which then prevented fibrosis and opacification of the lens. In brief, this study preliminarily discusses the microtubule-related regulation during lens repair.

吴 骏 简 介

吴骏，药学博士，副研究员，中国科学院动物研究所国家干细胞资源库研发部负责人。长期从事干细胞药物研究工作，在创新干细胞药物的研究开发、临床应用和产业转化工作中做出了重要的原创性工作。2020 年新冠肺炎疫情期间，带领团队紧急攻关研制全球首个具有自主知识产权的新冠肺炎干细胞药物——CAStem 细胞注射液，获得国家药品监督管理局 I/II 期药物临床试验批件，用于治疗重症新冠肺炎所致急性呼吸窘迫综合征和肺纤维化，入选国务院联防联控机制推荐的治疗新冠肺炎“三药三方案”。开展半月板损伤、原发性卵巢功能不全、中重度宫腔粘连、新冠肺炎等国家两委局备案干细胞临床研究 6 项，以及国家药监局批准 I/II 期药物临床试验 3 项。发表 SCI 论文 27 篇，在 Cell Res、Brit J Pharmacol、Cell Death Dis 等国际期刊发表一作/通讯作者论文 18 篇。申请专利 6 项，授权 1 项。起草并颁布干细胞相关团体标准 1 项。实现多项干细胞药物的临床应用和产业转化。

干细胞药物研发——从理论到实践

吴骏
国家干细胞资源库

【摘要】干细胞药物在重大疾病治疗、突发公共卫生事件应急响应中显示出巨大潜力，已经成为全球生物医药领域研发的前沿与热点，也是我国生命科技领域重点发展方向。截至目前，我国国家两委局备案的干细胞临床研究突破百项，国家药监局批准的注册干细胞药物临床试验超过二十项，但我国尚未有干细胞药物获批上市。不同于化学小分子药物、生物大分子药物是“死”的，具有均质、稳定等特性，干细胞药物是“活”的，具有很强的异质性和可塑性。因为成药研究对象的转变，干细胞药物对已有的传统药物研发体系带来了冲击，其产业化道路也面临着诸多挑战。多能干细胞具有无限增殖、自我更新和多向分化潜能的特性，理论上是干细胞药物的理想种子细胞。国家干细胞资源库建立了临床级人胚干细胞系以及临床级人胚干细胞实体库。经过多年干细胞药物研发的理论探索和实践创新，研制了多个具有自主知识产权的干细胞药物，开展了我国首个基于人胚干细胞的注册药物临床试验，并实现多项产业转化。

在线投稿摘要合集

A critical role of dental niche cells in tooth morphogenesis revealed by single cell sequencing and a dual color mouse model.

周波

中国科学院

【**Abstract**】For decades, tooth morphogenesis was known to be dependent on the inductive epithelial-mesenchymal interaction. However, the cellular basis for such reciprocal interaction was not yet known. To answer this, we developed a dual fluorescence reporter mouse to track and analyze developing dental epithelium and mesenchyme at single-cell resolution. To restore spatial information from scRNA-seq, a virtual molar explorer (VMEx) model was constructed that mapped 15,967 molar-expressed genes, where we identified that Msx1+ Sdc1+ marked the developing dental papilla while surrounded by Msx1+ Sdc1- molar niche. Through tooth germ reconstitution and organoid culture in vitro and transplantation in vivo, surprisingly, Msx1+ Sdc1- niche cells were found to function as the tooth organizers by promoting epithelium survival and tooth germ organization. The development and appearance of Msx1+ Sdc1+ dental papilla was a direct result of the inductive interaction between dental epithelium and Msx1+ Sdc1- niche, which was further confirmed in vivo. Together, our results revealed the cellular dynamics of tooth development in mice and identified the dental niche cells as the key driver of epithelial-mesenchymal interaction and tooth morphogenesis.

RNA 修饰稳态与白血病干细胞功能调控研究

张好建

武汉大学

【 **Abstract** 】 N6-methyladenosine (m6A) is a commonly present modification of mammalian mRNAs and plays key roles in various cellular processes. m6A modifiers catalyze this reversible modification. However, the underlying mechanisms by which these m6A modifiers are regulated remain elusive. Here we show that expression of m6A demethylase ALKBH5 is regulated by chromatin state alteration during leukemogenesis of human acute myeloid leukemia (AML), and ALKBH5 is required for maintaining leukemia stem cell (LSC) function but is dispensable for normal hematopoiesis. Mechanistically, KDM4C regulates ALKBH5 expression via increasing chromatin accessibility of ALKBH5 locus, by reducing H3K9me3 levels and promoting recruitment of MYB and Pol II. Moreover, ALKBH5 affects mRNA stability of receptor tyrosine kinase AXL in an m6A - dependent way. In addition, YBX1 interacts with insulin-like growth factor 2 messenger RNA (mRNA)-binding proteins (IGF2BPs) and stabilizes m6A-tagged RNA. YBX1 deficiency dysregulates the expression of apoptosis-related genes and promotes mRNA decay of MYC and BCL2 in an m m6A-dependent manner, which contributes to the defective survival that results from deletion of YBX1. Thus, our findings link chromatin state dynamics with expression regulation of m6A modifiers and uncover a selective and critical role of ALKBH5 and YBX1 in AML that might act as a therapeutic target of specific targeting LSCs.

干细胞模拟衰老相关疾病

邵玥
清华大学

【**Abstract**】Blood-borne factors capable of delaying aging in individual tissues are of interest as rejuvenation strategies, but how they achieve cellular and systemic level effects has remained unclear. Here, we constructed a single-cell transcriptomic map of aged organs and their rejuvenation in heterochronic parabiosis (HP), a classical model to study systemic aging. Among all cell types characterized in HP atlas, we identified hematopoietic stem and progenitor cells (HSPCs) as the most responsive ones to young blood exposure, from which a continuum of cell state and composition changes across the hematopoietic and immune system emanated. Exposure to young blood partially reversed age-associated lymphopoiesis decline and was associated with restoration of youthful transcriptional regulatory program and cytokine-mediated cell-cell communications in HSPCs. In the skin, stem cells were also revitalized, along with restored interactions with replenished endothelial niche cells. Overall, we revealed cellular and molecular programs that instruct systemic rejuvenation by blood-borne factors.

Capturing and Creating Patient Specific Liver Disease Avatars

Yun Shen Chan
生物岛实验室

【**Abstract**】Our knowledge of mammalian tissue and organ development has greatly increased over the years. Numerous signaling pathways, cellular processes, and transcriptional regulators that govern cell fate have been unraveled. This wealth of knowledge and the rapid advancement of cell culture tools and reagents have enabled the efficient isolation of primary cells from the human adult tissue and organs. We are witnessing a paradigm shift in the development of patient and organ-specific disease models with these tissue-derived primary cell lines including cancer studies across various indications. Our lab utilizes knowledge of how various liver cell types proliferate, and different cell culture methods to capture and create patient-specific models of liver diseases. In this talk, I will describe some of these ongoing efforts to generate hepatic stem cells from adult liver tissues and attempts to utilize these cell models to resolve challenges in modeling and understanding liver diseases.

Integrating GEMMs and Lineage Tracing to Study Pancreatic Cancer EMT and Metastasis

郑小凤
浙江大学

【**Abstract**】Diagnosis of pancreatic ductal adenocarcinoma (PDAC) is associated with dismal prognosis despite current therapies, therefore new treatment strategies are urgently required. Numerous studies have suggested that epithelial to mesenchymal transition (EMT) contributes to early-stage dissemination of cancer cells and is pivotal for invasion and metastasis of PDAC. EMT program is associated with phenotypic conversion of epithelial cells into mesenchymal-like cells in cell culture conditions, albeit such defined mesenchymal conversion (with spindle shaped morphology) of epithelial cells is rare with quasi-mesenchymal phenotypes occasionally observed in the tumor (partial EMT). Most studies exploring the functional role of EMT in tumors have depended on cell culture induced loss-of-function and gain-of-function experiments involving EMT inducing transcription factors such as Twist and Snail. Therefore, the functional contribution of EMT program for invasion and metastasis remains unclear and genetically engineered mouse models (GEMMs) to specifically address a causal connection are lacking. Here we functionally probed the role of EMT program in PDAC by generating PDAC GEMMs with deletion of Snail or Twist, two key transcription factors responsible for EMT. EMT suppression in the primary tumor did not alter the emergence of invasive PDAC, systemic dissemination and metastasis. Suppression of EMT led to an increase in cancer cell proliferation with enhanced expression of nucleoside transporters in tumors, contributing to enhanced sensitivity to gemcitabine treatment and increased overall survival of mice. Furthermore, mesenchymal cell reporter mice driven by aSMA-Cre and Fsp1-Cre with genetically engineered mice that develop spontaneous pancreatic ductal adenocarcinoma (PDAC) were employed to monitor partial EMT program. The established metastases were primarily composed of cancer cells without evidence for a partial EMT program, as assessed by our fate mapping approach. In contrast, metastatic cancer cells exhibiting a partial EMT program were restricted to isolated single cancer cells or micrometastases (3–5 cancer cells). Collectively, our study suggests that Snail or Twist induced EMT program is not rate-limiting for invasion and metastasis but highlights the importance of combining EMT inhibition with chemotherapy for the treatment of pancreatic cancer. And further study identifies large metastatic nodules with preserved epithelial phenotype and potentially unravel a novel metastasis program in PDAC.

乳腺癌潜伏干细胞的生存机制

蔡尚

西湖大学

【摘要】乳腺癌患者在接受肿瘤根治术和辅助治疗后的数年甚至数十年间，仍然存在远处器官转移的风险。尽管有证据提示属于少数亚群的肿瘤干细胞能够长期休眠于靶器官，并最终形成转移瘤，但机体的免疫防御机制仍然无法彻底清除潜伏的肿瘤干细胞，从而完全排除转移复发的可能性。利用自发性乳腺癌伴肺转移模型，我们从单细胞转录组角度对具有转移起始功能的乳腺癌干细胞进行了信息挖掘，筛选出特异膜蛋白作为潜伏肿瘤干细胞标记物，并探讨了膜蛋白在乳腺癌干细胞转移潜伏中通过抑制天然免疫系统从而逃避监视的重要作用及信号机制。药物抑制实验表明，可以有效阻碍乳腺癌在肺组织早期潜伏与晚期结节形成。此研究为全面清除潜伏在其他脏器的乳腺癌干细胞提供了有价值的靶点及途径。

单细胞测序与视网膜再生

鲁岩

中山大学附属第三医院

【摘要】斑马鱼能通过重编程米勒胶质细胞从而完全实现视网膜的损伤修复，小鸡能部分实现视网膜损伤修复，而哺乳动物不能。这背后所涉及的核心调控机制并不清楚。运用单细胞转录组测序（Single-cell RNA-Seq）和表观调控组测序技术（ATAC-Seq）我们分别检测了斑马鱼、小鸡和小鼠在视网膜损伤后米勒细胞的基因表达和染色体开放性的变化。通过单细胞测序分析我们在斑马鱼和小鸡中确定了米勒细胞损伤响应的三种状态：静息、激活和增生。相应的，我们通过新的计算方法重构了反映不同米勒细胞状态的调控网络。研究发现在激活状态下高表达的转录因子如斑马鱼的 yap1，hmga1 或小鸡的 FABP 转录因子，非常关键地调控着斑马鱼和小鸡米勒细胞的重编程。敲除这些转录因子完全抑制了米勒细胞的增生。在小鼠中，一组特异的调控网络抑制着米勒细胞的重编程，包括转录因子 NFI。敲除 NFI 后，小鼠的米勒细胞重编程为视神经细胞。通过基因调控网络分析我们提出了斑马鱼和小鼠的米勒细胞损伤响应的不同调控模型，这解释了为什么斑马鱼可以完全实现视网膜再生修复而哺乳动物不行。同时该研究也有助于通过靶向核心调控网络实现对神经退行性疾病的治疗。

外周血 MSC 用于肌肉骨骼系统组织再生的基础研究及其临床应用

付维力

四川大学华西医院

【摘要】我们首先报道散打运动员滑车区全层软骨缺损的病例报道。因为该临床病例的成功报道，开展系列的外周血 MSC 的体外生物学特性研究及探索其在肌肉骨骼系统组织再生中的应用：1.建立稳定的外周血 MSC 动员分离培养及鉴定的方法，全面比较外周血和骨髓来源的 MSC 的细胞生物学特性，并复合脱钙皮质骨修复兔关节软骨缺损发现两种来源 MSC 体内促进软骨再生的能力相似；2.外周血 MSC 与 EPC 的共培养及其复合掺锶聚磷酸钙（SCPP）构建血管化工程骨的研究；3. BMP-12 重组腺病毒载体转染外周血 MSC 向肌腱/韧带细胞分化研究；4.半月板细胞与外周血 MSC 体外二维和三维条件下共培养体系的建立促进去分化的半月板纤维软骨细胞的复分化。所有这些研究旨在探索外周血干细胞的进一步应用：外周血来源 MSC 较其它来源取材更微创，获取过程中的并发症更少，并且可以完全实现自体移植的目标。目前大多数医院都有外周血干细胞移植的临床治疗项目，临床推广的可行性很大。

【关键词】 外周血 MSC；肌肉骨骼系统组织再生；临床应用

Human Wharton's Jelly MSC-Derived Small Extracellular Vesicles：A Naturally Nanotherapeutic Agent Ameliorates Osteoarthritis by Carrying miRNA Cargo

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【**Abstract**】Background:Extracellular vesicle, an a cell-free nanotherapeutic agent have robust anti-inflammatory and regeneration effects in degenerative joint diseases, which can effectively surmount many obstacles and limitations derived from mesenchymal stem cell-based therapy or synthetic nanoparticles. Here, our study aimed to investigate the therapeutic effects and the molecular mechanism of Wharton's jelly mesenchymal stem cell-derived extracellular vesicle (WJMSC-EVs) in osteoarthritis (OA).

Methods:The WJMSC-EVs were isolated by size-exclusion chromatography (SEC) for identification by morphology observation, including scanning electron microscope (SEM), transmission electron microscopy and atomic force microscopy, particle size analysis, including dynamic light scattering, nanoparticle tracking analysis, nano-flow cytometry, surface markers detection including western blot and flow cytometry. In vitro, IL-1β-induced OA chondrocytes were established to evaluate cell proliferation and migration by CCK-8 assay and transwell assay, respectively. Meanwhile, OA-related and macrophage-related genes expression was quantified using real-time PCR. In vivo, a rat OA model were induced by transecting anterior cruciate ligament, micro-CT, histological and immunohistochemistry analyses were used to assess the efficacy of WJMSC-EVs and WJMSCs. Meanwhile, small miRNA-seq was used to analyze the expression profiles of exosomal miRNAs derived from WJMSC-EVs, thereby probing into the protective mechanism of WJMSC-EVs on chondrocytes.

Results:WJMSC-EVs has an typical discs morphology with a diameter of approximately 100nm. The WB results confirmed that the WJMSC-EVs highly expressed CD9, TSG101 and CD63 but did not express the EV-negative protein marker calnexin, which were met the typical criteria for EVs. WJMSC-EVs successfully fused into chondrocyte in vitro, and attenuated IL-1β-induced chondrocyte proliferation and migration inhibition. WJMSC-EVs increased chondrogenic genes Col2A1, decreased MMP-13 and ADAMTS-5. Furthermore, WJMSC-EVs promoted macrophage polarization toward the M2 phenotype. In the animal study, both of WJMSCs and WJMSC-EVs treatment significantly upregulated Collagen II and downregulated MMP-13 protein and ADAMTS-5 protein in the cartilage tissue of the OA rat. Moreover, WJMSC-EVs treatment promote subchondral bone reconstruction similar to WJMSCs. Of note, we found that many potent miRNAs abundant in WJMSC-EVs has been confirmed to can promote cartilage regeneration through activating PI3K/Akt, NOTCH and Hippo pathway.

Conclusion:WJMSC-EV-based nanotherapeutics possess great biocompatibility and can effectively protect against cartilage erosion. This effect may be attributed to inhibiting

inflammation, regulating immune and maintaining a healthy microenvironment by miRNAs. In summary, WJMSC-EV may constitute a novel potential therapeutic strategy for OA.

【**Keywords**】 miRNAs, nanotherapeutic agent, osteoarthritis, regeneration, Wharton' s jelly mesenchymal stem cells derived small extracellular vesicles

Comparison of the Response to the CXCR4 Antagonist AMD3100 during Development of Retinal Organoids Derived from ES Cells and of Zebrafish Retina

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【**Abstract**】Retinal organoids generated from embryonic stem cells or iPSCs recreate the key structural and functional features of mammalian retinal tissue in vitro. However, the differences in development of retinal organoids and normal retina in vivo are not well defined. Previous studies have demonstrated that C-X-C chemokine receptor type 4 (CXCR4) plays a key role in neurogenesis and optic nerve development in the retina. Thus, in the present study, we analyzed the development of retinal organoids and zebrafish retina after inhibition of CXCR4 with the antagonist AMD3100. Our data indicated that CXCR4 was mainly expressed in ganglion cells in retinal organoids and was not expressed in amacrine or photoreceptor cells. AMD3100 treatment reduced the retinal organoid generation ratio by approximately 40% and induced abnormal morphological changes. Moreover, CXCR4 blockade impaired differentiation of retinal cells in the organoids. The numbers of ganglion cells, amacrine cells, and photoreceptors were decreased by approximately 25-30% in organoids treated with AMD3100 compared to those in the control. Abnormal locations of ganglion, amacrine, and photoreceptor cells were observed in organoids treated with AMD3100. Neuronal axon outgrowth was also damaged in retinal organoids. Most results were similar to the data obtained in zebrafish retina. A decrease in ganglion cells, amacrine cells, and photoreceptors and the distribution of neural outgrowth induced by AMD3100 treatment in zebrafish retina were consistent with those detected in organoids, except that photoreceptors were not mislocalized. Abnormal photoreceptor ensembles, such as ‘rosettes’, induced by AMD3100 treatment in the organoids were not detected in zebrafish retina. Therefore, our study suggests that retinal organoids may be a reliable model for reproduction of retinal development; however, certain differences between organoids and the retina in vivo were detected.

【**Keywords**】Retinal organoids; Retinal developmental comparison; zebrafish; C-X-C chemokine receptor type 4 (CXCR4);

Enhanced HSC-like cell generation from mouse pluripotent stem cells in a 3D induction system cocultured with stromal cells

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【**Abstract**】Background: Decades of efforts have attempted to differentiate the pluripotent stem cells (PSCs) into truly functional hematopoietic stem cells (HSCs), yet the problems of low differentiation efficiency in vitro and poor hematopoiesis reconstitution in vivo still exist, mainly attributing to the lack of solid, reproduced or pursued differentiation system.

Methods: In this study, we established an in vitro differentiation system yielding in vivo hematopoietic reconstitution hematopoietic cells from mouse PSCs through a 3D induction system followed by coculture with OP9 stromal cells. The in vivo hematopoietic reconstitution potential of c-kit+ cells derived from the mouse PSCs was evaluated via m-NSG transplantation assay. Flow cytometry analysis, RNA-seq and cell cycle analysis were used to detect the in vitro hematopoietic ability of endothelial protein C receptor (EPCR, CD201) cells generated in our induction system.

Results: The c-kit+ cells from 3D self-assembling peptide induction system followed by the OP9 coculture system possessed apparently superiority in terms of in vivo repopulating activity than that of 3D induction system followed by the 0.1% gelatin culture. We interestingly found that our 3D+OP9 system enriched a higher percentage of CD201+c-kit+cells that showed more similar HSC-like features such as transcriptome level and CFU formation ability than CD201-c-kit+cells, which haven't been reported in the field of mouse PSCs hematopoietic differentiation. Moreover, CD201+ hematopoietic cells remained in a relatively slow cycling state, consistent with high expression levels of P57 and Ccng2. Further, we innovatively demonstrated that notch signaling pathway is responsible for in vitro CD201+ hematopoietic cell induction from mouse PSCs.

Conclusions: Altogether, our findings lay a foundation for improving the efficiency of hematopoietic differentiation and generating in vivo functional HSC-like cells from mouse PSCs for clinical application.

【**Keywords**】Keywords: 3D system, Pluripotent stem cells, Hematopoiesis, CD201, Notch

脂肪干细胞外泌体通过抑制 MITF/cAMP 通路抑制黑色素产生

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【摘要】目的：随着大众审美的改变，市场对美白产品的追求热度只增不减。近几年随着自体脂肪移植的开展，尤其是面部填充方面，临床医生发现填充者的面部皮肤的状态、颜色发生变化。已有相关文献证明脂肪干细胞的上清液能够抑制黑色素形成，并且市场上有相关产品上市。外泌体近几年来引起了研究热潮，其在再生医学、骨关节炎及眼科发挥着巨大的作用。脂肪作为来源最广的组织之一，其外泌体较其他位置丰富，作为脂肪上清液的成份之一，其对黑色素的作用应值得探讨。

方法：1.使用递增浓度的外泌体浓度对小鼠黑色素瘤细胞及人表皮黑色素细胞作用 48h，观察黑色素的抑制效果。2.使用递增浓度的外泌体浓度预处理小鼠黑色素瘤细胞及人表皮黑色素细胞作用 1h，再用黑色素刺激生成素作用 24h。分别测量黑色素含量、酪氨酸酶活性、酪氨酸酶相关表达基因以及 MITF 的变化。使用递增浓度的外泌体浓度对斑马鱼卵进行体内实验。观察胚胎生长情况。

结果：脂肪干细胞外泌体通过调控 MITF 的表达下调抑制黑色素的形成。

结论：相对于等量脂肪干细胞上清液，脂肪干细胞外泌体的对黑色素生成的抑制效用更大。

【关键词】 脂肪干细胞 黑色素

Mesenchymal stem cell carriers enhance antitumor efficacy induced by oncolytic reovirus in acute myeloid leukemia

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【**Abstract**】Chemotherapy is the main treatment for acute myeloid leukemia (AML), but the therapeutic efficacy is modest, and most commonly manifests as relapse from remission. Thus, improving long-term AML survival is a crucial clinical challenge. In recent years, oncolytic virotherapy has provided an alternative approach for AML treatment. The use of oncolytic reoviruses has been explored in more than 30 clinical trials for safety and feasibility issues. However, like other oncolytic viruses, neutralizing antibodies (NAbs) reduce therapeutic efficacy. To tackle this problem, human umbilical cord mesenchymal stem cells (hUC-MSCs) were used to deliver reovirus using in vitro and in vivo models. Human UC-MSCs were successfully loaded with reovirus, without impairing biological function. We also observed in vitro protective effects of hUC-MSCs on reovirus in the presence of NAbs. In the immunocompromised AML mouse model, hUC-MSCs effectively carried reoviruses to tumor lesions and significantly prolonged the survival of AML xenografts in mice in the presence of a high titer anti-reovirus antibody ($p=0.001$). However, reovirus-induced activation of AKT, stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and NF-κB signaling led to the maintenance of intrinsic migratory properties and secretion of pro-inflammatory cytokines from hUC-MSCs, particularly CXCL10. In immuno-competent AML mice, MSCs carrying reovirus triggered immune responses, and eventually inhibited tumor growth. Therefore, these results suggest that MSCs as carriers of oncolytic reoviruses can enhance the antitumor efficacy of virotherapy.

【**Keywords**】 Human umbilical cord mesenchymal stem cells, Oncolytic reovirus, Acute myeloid leukemia, Antitumor efficacy, CXCL10, Virotherapy

Human umbilical cord derived mesenchymal stem cells transfer oncolytic reovirus to tumor cells via extracellular vesicle release

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【**Abstract**】Oncolytic viruses are powerful novel agents for suppressing tumor growth. Similar to other oncolytic viruses, reovirus is exposed to antiviral immunity in vivo when administered intravenously, which greatly reduces the anti-tumor efficacy. To overcome this obstacle, human umbilical cord mesenchymal stem cells (UC-MSCs) were used as cellular vehicles to deliver oncolytic reovirus. Transmission electron microscopy and indirect immunofluorescence staining were used to verify the presence of reovirus in the cytoplasm of UC-MSCs. Transwell co-culture experiments showed that reovirus released by UC-MSCs was able to pass through a 0.4 μm porous membrane and infect tumor cells in the presence of neutralizing antibodies. The virus was detected in extracellular vesicles (EVs) derived from pre-infected UC-MSCs, and virus-encapsulating EVs had a killing effect on tumor cells. Collectively, the results indicate that UC-MSCs transfer reovirus to tumor cells via EV release, thus, providing a theoretical basis for the use of UC-MSCs as carriers of oncolytic reovirus.

【**Keywords**】Human umbilical cord-derived mesenchymal stem cells, oncolytic reovirus, transfer, extracellular vesicle, neutralizing antibodies

Smurf1 对人类颅缝间充质干细胞成骨分化能力的影响

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【摘要】目的 探讨 smurf1 对人类颅缝间充质干细胞成骨分化能力的影响。方法 首先在体外进行颅缝组织外植体分离培养人颅缝细胞，并通过流式细胞术及多系分化能力分别鉴定人类颅缝来源细胞的间充质干细胞特性，进一步通过实时实时定量 PCR 检测颅缝间充质干细胞中成骨相关基因的表达水平。转染 smurf1 过表达及干扰慢病毒观察其对颅缝间充质干细胞成骨分化能力的影响。结果 体外成功培养出人类颅缝衍生细胞，通过流式细胞学分析显示该类细胞高度表达间充质干细胞标志，同时可向三系分化。通过茜素红染色显示早闭侧颅缝成骨分化能力强于正常侧细胞，同时，调控成骨分化相关基因 OCN 及 Runx2 基因在早闭侧间充质干细胞中表达较另一组细胞明显上调。实时定量 PCR 发现早闭侧间充质细胞较正常侧低表达 smurf1 基因，转染过表达 Smurf1 慢病毒后通过泛素化降解 BMPR1A 抑制 BMP 通路使早闭侧颅缝间充质干细胞成骨分化能力下降。然而，在正常侧细胞中转染了 Smurf1-shRNA 慢病毒后，与对照组相比较，smurf1 基因及蛋白水平显著下调，BMPR1A 蛋白水平上升，其成骨分化能力明显增加。结论 通过抑制 smurf1 的表达有效激活 BMP 通路，可增强颅缝间充质干细胞的成骨分化能力，从而导致颅缝早闭症的发生。不仅如此，深入理解颅缝间充质干细胞特性可以为我们提供在颅骨缺损修复及颅缝再造等再生医学中的细胞生物学基础。

【关键词】 颅缝；间充质干细胞；成骨分化；颅缝早闭

Effective Control of Large Deletions After Double-Strand Breaks by Homology-Directed Repair and dsODN Insertion

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【**Abstract**】Background: After repairing double-strand breaks (DSBs) caused by CRISPR-Cas9 cleavage, genomic damage, such as large deletions, may have pathogenic consequences. Results: We show that large deletions are ubiquitous but dependent on editing sites and cell types. Human primary T cells display more significant deletions than hematopoietic stem and progenitor cells (HSPCs), whereas we observe low levels in induced pluripotent stem cells (iPSCs). We find that the homology-directed repair (HDR) with single-stranded oligodeoxynucleotides (ssODNs) carrying short homology halves the deletion damage, while adeno-associated virus (AAV) donors with long homology reduce large deletions by ~80%. In the absence of HDR, the insertion of a short double-stranded ODN by NHEJ at DSBs reduces deletion indexes by ~60%.

Conclusions: Timely bridging of broken ends by HDR and NHEJ vastly decrease the unintended consequences of dsDNA cleavage. These strategies can be harnessed in gene editing applications to attenuate unintended outcomes.

【 **Keywords** 】 CRISPR-Cas9, genome editing, large fragment deletions, nanopore sequencing, homology-directed repair (HDR), Nonhomologous end joining (NHEJ), T cells, hematopoietic stem and progenitor cells (HSPCs), induced pluripotent stem cells (iPSCs)

Modeling metabolic disease and drug response using patient stem cells

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【**Abstract**】Glucocorticoids (GCs) are widely used as anti-inflammatory drugs, but their long-term use has severe metabolic side effects. Here, by treating multiple individual adipose stem cell-derived adipocytes and induced pluripotent stem cell-derived hepatocytes with the potent GC dexamethasone (Dex), we uncovered celltype- specific and individual-specific GC-dependent transcriptomes and glucocorticoid receptor (GR) cistromes. Individual-specific GR binding could be traced to single-nucleotide polymorphisms (SNPs) that altered the binding motifs of GR or its cooperating factors. We also discovered another set of genetic variants that modulated Dex response through affecting chromatin accessibility or chromatin architecture. Several SNPs that altered Dex-regulated GR binding and gene expression controlled Dex-driven metabolic perturbations. Remarkably, these genetic variations were highly associated with increases in serum glucose, lipids, and body mass in subjects on GC therapy. Knowledge of the genetic variants that predispose individuals to metabolic side effects allows for a precision medicine approach to the use of clinically relevant GCs.

【**Keywords**】Stem cell, metabolic disease, precision medicine, transcriptional regulation

类脊髓组织构建及其移植修复脊髓损伤的研究进展

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【摘要】近年来，中枢神经的大脑类器官引起了人们极大的兴趣，因其有潜力在不受动物模型限制下研究大脑发育以及病理学大脑类器官具有细胞类型的多样性、神经网络的健全度以及成熟神经元的结构等特点。中枢神经的另一个器官类脊髓研究，则较少有文献报道。在类脊髓器官研究方面，我们团队在体外成功构建了一种可用于移植修复全横断脊髓损伤的类脊髓组织，并发现该组织移植后能够整合到宿主脊髓神经网络中，实现动物瘫痪后肢的重新站立和支撑体重。

我们综合性应用神经干细胞、神经营养因子和生物材料，在三维培养体系中，经过一定时间的动态相互作用形成类脊髓组织。初步的表型和基因型检测显示，该组织含有类似于正常脊髓白质和灰质结构及其主要组织细胞(神经元、少突胶质细胞和星形胶质细胞)及细胞外基质，即由白质模块和灰质模块组合而成。该研究结果揭示，类脊髓组织的白质模块和灰质模块通过动态的相互作用最终实现功能性整合。这种工程化新技术构建的类脊髓组织，为修复严重性脊髓损伤后组织结构和功能提供了可能，因此具有较好的应用前景。下一步研究将证实类脊髓组织移植能够按照脊髓白质对应白质、灰质对应灰质的配对模式整合到全横断脊髓缺损区，更高效地发挥工程化类脊髓组织的中继作用(neuronal relay)，接收、协调和传导上、下行神经环路信息，从而提升全横断脊髓损伤后瘫痪肢体的自主运动和感觉功能。此外，工程化类脊髓组织还有望作为体外神经类药物的高通量筛选平台

【关键词】 类器官; 类脊髓组织; 神经干细胞; 神经营养因子; 生物材料; 细胞外基质; 移植; 脊髓损伤; 修复; 神经环路; 运动功能

Cell fate roadmap of human primed-to-naive transition reveals preimplantation cell lineage signatures

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【 **Abstract** 】 Human naive pluripotent stem cells offer a unique window into early embryogenesis studies. Recent studies have reported several strategies to obtain cells in the naive state. However, cell fate transitions and the underlying mechanisms remain poorly understood. Here, by a dual fluorescent reporter system, we depict the cell fate dynamics from primed state towards naive pluripotency, with ALPG activation as a hallmark. Integration of transcription profiles and the chromatin accessibility landscape reveal the transitioning cells branching into primitive endoderm- and trophectoderm-like subpopulations, with the capacities for derivation of extra-embryonic endoderm and trophoblast stem cell lines, respectively. Furthermore, despite different fluorescent dynamics, all transitioning intermediates are capable of reaching the naive state with prolonged induction, showing their developmental plasticity and potential. Overall, our study describes a global cell roadmap towards naive pluripotency, deciphers the key factors important for cell fate branching into preimplantation lineages, and provides hints for embryo modeling-related studies.

【 **Keywords** 】 human naive pluripotency, primed-to-naive state transition, cell fate dynamics, preimplantation lineage signatures

Muse 成体干细胞对角膜损伤的修复再生

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【摘要】Muse 干细胞因其阳性表达多能干细胞和间充质干细胞的标记物而又具备非致瘤性的特性被认为是未来临床应用的重要种子细胞，目前已见大量报道称 Muse 干细胞在动物模型移植后可自发迁移至损伤部位并分化为损伤细胞并替代其行使功能，从而促进如心脏、肝脏, 肺等组织的再生。然而，目前并未见有报道关于 Muse 干细胞在眼睛或角膜损伤修复方面的研究。因此，本研究的主要目的是探索应用 Muse 干细胞分化来源的 CSCs（M-CSCs）治疗小鼠和树鼩的角膜基质损伤瘢痕。我们首次证明了 3D 培养可以促进 Muse 干细胞的增殖，增加 Muse 干细胞的干性并保持其特性；此外我们还证明 Muse 干细胞可以分化为功能性的 CSC，并且 Cell-SCC 的移植可以有效阻止小鼠和树鼩角膜损伤瘢痕的形成，减少损伤角膜的炎症反应和新生血管的形成，促进损伤角膜神经的再生。

【关键词】 角膜瘢痕, 树鼩, 角膜基质细胞，Muse cells,

人类胚胎干细胞的细胞周期中染色质高维结构的维持机制研究

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【**Abstract**】During S phase, genomic DNA is replicated in a defined temporal order named replication timing(RT). All eukaryotes have an RT program, which has been reported to be highly correlated with epigenetic traits such as 3D genome organization including TADs and compartments. It still remains unknown whether RT helps maintaining higher order chromatin organization during cell cycle. Here we disrupt RT globally by auxin induced degradation of RIF1 in human embryonic stem cells (hESCs) to see whether 3D genome organization is affected differentially in heterochromatin and euchromatin which is marked by H3K9me3 and H3K27ac respectively. This finding is important for understanding mechanisms for maintaining genome organization in pluripotent stem cells.

【**Keywords**】TADs；compartments； hESCs；H3K9me3；replication timing

基于液滴微流控的模块化组织 3D 打印技术

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大连理工大学

【摘要】实现单细胞精度、高细胞密度的工程化类组织精准制造将在组织/器官损伤修复和疾病治疗方面有着巨大应用潜力。本研究提出以微凝胶（Microgels）组装的颗粒水凝胶（Granular hydrogels）作为 3D 生物打印墨水，基于液滴微流控（Droplet microfluidics）技术精准设计制造载单细胞微凝胶载体作为基本模块，通过微颗粒组装和 3D 打印技术结合突破单细胞精度的仿生组织体外精准构建。

【关键词】 载细胞微凝胶；微流控液滴技术；3D 生物打印；模块化组织工程；干细胞

多能干细胞来源的肾脏类器官的建立及应用

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【摘要】肾脏作为重要的人体器官，具有排除体内代谢废物，调节电解质平衡，分泌肾素、促红细胞生成素等作用，并参与机体其他生理功能。然而出生以后，肾脏的再生功能逐渐下降。受到衰老、疾病、环境等因素的影响，肾脏结构功能发生改变，逐步发展为慢性肾脏疾病（chronic kidney disease，CKD）。2020 年《柳叶刀》发表的研究发现全球共有 6.975 亿 CKD 患者。而其中，中国患病人数高达 1.32 亿。CKD 是导致终末期肾病（end-stage renal disease ，ESRD)的主要原因之一。而病人一旦发展为 ESRD，则需要进行肾脏替代疗法，即腹腔透析、血液透析、或者肾移植。巨大的透析费用和有限的肾源供应，限制了该疾病的治疗。因此，建立一种接近人体肾脏结构功能的模型，来研究肾脏发育机制，探索肾脏疾病的致病机理，寻找潜在的可替代移植性肾源是非常有必要的。多能干细胞定向诱导分化建立的肾脏类器官为研究肾脏早期发育机制和肾脏疾病机理提供了一个全新的平台。本课题组建立了一个高效稳定的肾脏分化系统，通过调节 WNT、TGF、FGF 信号通路，将人多能干细胞定向诱导分化，经过原条、中间中胚层、肾脏祖细胞阶段，最终得到肾单位的相关结构，包括足细胞、近端小管、远端小管等结构。另外，我们得到的肾脏类器官还包含丰富的血管内皮细胞网络系统。利用本分化平台，我们将多囊肾病人来源的 iPSC 进行诱导分化，建立多囊肾类器官，为多囊肾致病机理以及药物筛选提供了全新的平台，初步进行了基于肾脏类器官的应用性研究。

【关键词】 多能干细胞，诱导分化，肾脏类器官，多囊肾

Cultrex™ UltiMatrix BME – One For All 类器官和干细胞培养的多用途 BME

Sol Degese、Xi Lu、David Galitz、Susan Tousey、Kevin Flynn

Bio-Techne

【摘要】类器官和干细胞培养技术的不断发展增加了我们对发育生物学的基本了解，并提高了体外疾病建模成功率和药物筛选的可预测性。小鼠 EHS 肿瘤的基底膜提取物（BME）制作的基质胶可用于和多能干细胞的扩增。这些 BME 的质量和批间差会大大影响模型的可变性。本研究介绍了一种先进的 BME，旨在克服现有的 EHS 来源基质胶的缺点。

Cultrex™ UltiMatrix 低生长因子（RGF）BME 是一种可溶形式的基质胶，具有高张力强度、高巢蛋白水平、高的蛋白质浓度的三高特性及稳定的透明度和纯度。这些成分上的改进可转化为显著的性能优势，使 Cultrex UltiMatrix RGF BME 成为一种理想的干细胞和类器官培养的基质胶。

【关键词】 类器官培养

Infusion of hESC derived Immunity-and-matrix regulatory cells improves cognitive ability in early-stage AD mice

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【**Abstract**】 Objectives: In this study, we administered immunity-and-matrix-regulatory cells (IMRCs) via tail vein (IV) and intracerebroventricular (ICV) injection to 3-month-old 5×FAD transgenic mice to assess the effects of IMRC transplantation on the behavior and pathology of early-stage Alzheimer's disease (AD).

Materials and Methods: Clinical-grade human embryonic stem cell (hESC)-derived IMRCs were produced under good manufacturing practice (GMP) conditions. Three-month-old 5×FAD mice were administered IMRCs via IV and ICV injection. After 3 months, the mice were subjected to behavioral tests and electrophysiological analysis to evaluate their cognitive function, memory ability, and synaptic plasticity. The effect of IMRCs on amyloid beta (Aβ)-related pathology was detected by thioflavin-S staining and western blot. Quantitative real-time PCR, ELISA, and immunostaining were used to confirm that IMRCs inhibit neuroinflammation. RNA-seq analysis was performed to measure changes in gene expression and perform a pathway analysis in response to IMRC treatment.

Results: IMRC administration via tail vein injection significantly ameliorated cognitive deficits in early-stage AD (5×FAD) mice. However, no significant change was observed in the characteristic pathology of AD in the ICV group. Plaque analysis revealed that IMRCs did not influence either plaque deposition or BACE1 expression. In addition, IMRCs inhibited inflammatory responses and reduced microglial activation in vivo.

Conclusions: We have shown that peripheral administration of IMRC can ameliorate AD pathology and associated cognitive deficits.

【**Keywords**】Immunity-and-matrix regulatory cells， Alzheimer，Clinical-grade human embryonic stem cell，Amyloid beta

An immunometabolic patch facilitates mesenchymal stromal/stem cell therapy for myocardial infarction through a macrophage-dependent mechanism

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【**Abstract**】 Mesenchymal stromal/stem cells (MSCs) have emerged as a promising approach to treat myocardial infarction. However, poor retention of transplanted cells in infarcted hearts significantly impedes their further clinical applications. Here, we showed that the administration of an inhibitor of glycolysis, 2-deoxy-D-glucose (2-DG), blocked the hyperinflammatory response within the ischemic myocardium and subsequently extended effective retention of transplanted MSCs. Mechanistically, 2-DG blocked the proinflammatory polarization of macrophages and suppressed the production of inflammatory cytokines. Selective macrophage depletion abrogated this curative effect. Finally, to avoid potential organ toxicity caused by systemic inhibition of glycolysis, we developed a novel chitosan/gelatin-based 2-DG patch that directly adhered to the infarcted region and facilitated MSC-mediated cardiac healing with undetectable side effects. This study pioneered the application of an immunometabolic patch in MSC-based therapy and provided insights into the therapeutic mechanism and advantages of this innovative biomaterial.

【**Keywords**】 Immunometabolism, Patch, Mesenchymal stromal/stem cells, Myocardial infarction, Macrophages, Glycolysis

Shh and Olig2 sequentially regulate oligodendrocyte differentiation from hiPSCs through PPARγ-mediated phospholipogenesis for the treatment of ischemic stroke

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【**Abstract**】Demyelination is a major component of white matter injury, characterized by myelin sheath loss and oligodendrocyte death, and results in memory loss and cognitive impairment in ischemic stroke. Increasing evidence shows that oligodendrocyte progenitor cells (OPCs) can be generated by the direct activation of defined transcription factors (TFs) from human induced pluripotent stem cells (hiPSCs). Here, we developed a stable, chemically defined protocol to generate OPCs as a cell therapy for ischemic stroke by partially inhibiting sonic hedgehog (Shh) activity transiently and selectively with GANT61 during neural induction from hiPSCs and sequentially inducing the overexpression of the TF Olig2. GANT61 treatment abolished motor neuron (MN) production by blocking Olig2 phosphorylation at Ser147 and thereby contributing to the MN-oligodendrocyte fate switch during neural induction. The early NG2+ OPCs was rapidly generated through sequentially collaborating with induced expression of Olig2 from neural progenitor cell -derived from hiPSCs. Mechanistically, Olig2-OPCs increased gene expression of the PPARγ signaling pathway-related gene expression, activated CEPT1-mediated phospholipogenesis. Functionally, inhibiting PPARγ and knocking down CEPT1 further promoted the terminal differentiation of Olig2-OPCs. Most importantly, when transplanted into a rat model of transient middle cerebral artery occlusion (tMCAO) along with and myelin disruption, Olig2-OPCs efficiently promoted neurological functional recovery by inhibiting neuronal apoptosis, promoting myelin reconstruction, and rescuing spatial memory decline during long-term recovery. We showed that Shh inhibition and forced Olig2 overexpression sequentially promoted the generation of human oligodendrocytes from hiPSCs, and that Olig2-OPC transplantation might be an ideal alternative approach for ischemic stroke rehabilitation.

【**Keywords**】 Olig2; sonic hedgehog (Shh); Oligodendrocyte progenitor cells (OPCs); Human induced pluripotent stem cells (hiPSCs); Ischemic stroke.

CHD8 safeguards early neuroectoderm differentiation in human ESCs and protects from apoptosis during neurogenesis

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【**Abstract**】 The chromatin remodeler CHD8, which belongs to the ATP-dependent chromatin remodelers CHD family, is one of the most high-risk mutated genes in autism spectrum disorders. However, the role of CHD8 in neural differentiation and the mechanism of CHD8 in autism remains unclear, despite there are a few studies based on the CHD8 haploinsufficient models. Here, we generate the CHD8 knockout human ESCs by CRISPR/Cas9 technology and characterize the effect of loss-of-function of CHD8 on pluripotency maintenance and lineage determination by utilizing efficient directed differentiation protocols. The results show loss-of-function of CHD8 does not affect human ESCs maintenance although having slight effect on proliferation and cell cycle. Interestingly, CHD8 depletion results in defective neuroectoderm differentiation, along with severe cell death in neural progenitor stage. Transcriptome analysis also indicates CHD8 does not alter the expression of pluripotent genes in ESC stage, but in neural progenitor cells depletion of CHD8 induces the abnormal expression of the apoptosis genes and suppresses neuroectoderm-related genes. These results provide the evidence that CHD8 plays an essential role in the pluripotency exit and neuroectoderm differentiation as well as the regulation of apoptosis during neurogenesis.

【**Keywords**】 CHD8, neuroectoderm, human ESCs, cell apoptosis

皮肤类器官及其微环境在疑难重症与罕见病治疗中的作用

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【摘要】干细胞龛位是如何通过调节干细胞的行为来维持组织内环境平衡和控制伤口愈合的，目前还没有完全弄清楚。我们通过组织工程技术结合激光捕获显微切割技术和空间定量蛋白质组学策略，成功获取了稳态和疾病状态下完整的皮肤干细胞龛成分，发现一种表皮干细胞龛（EpSCs' niches）的新基质蛋白，可促进表皮干细胞的生长和功能，以及伤口愈合过程。我们利用该蛋白成功治疗了一种皮肤罕见病—显性营养不良性表皮松解症（DDEB）。该蛋白能够成功恢复患者体细胞重编程 hiPSC 诱导分化的表皮干细胞干性和功能失调，并能促进 DDEB 小鼠难愈性伤口的再表皮化过程。我们的结果为干细胞龛位的蛋白质如何支持表皮干细胞的生长和功能提供了见解。此外，我们利用该蛋白建立了人诱导多能干细胞（hiPSC）衍生的具有毛囊（HFs）和神经系统（NS）的皮肤类器官模型，并研究了类器官对 SARS-CoV-2 感染的易感性。同时，我们利用皮肤类器官治疗另外一种皮肤罕见病（硬皮病），实现了该疾病萎缩的表皮附属器和血管的新生。进一步，我们通过构建出生缺陷的罕见病皮肤类器官结合多组学技术（蛋白质组学、单细胞测序等），从细胞内、外环境研究该疾病在生理和病理发育过程中的细胞和蛋白层级的致病机制，并推动类器官在罕见病治疗和药物筛选中的应用。

【关键词】 表皮干细胞，干细胞龛位，类器官，疑难重症与罕见病，空间蛋白质组学

探究微载体对高剂量卡铂所致血小板减少的保护作用

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【摘要】卡铂为一种广谱抗肿瘤药，其稳定性好，肿瘤杀伤作用强，应用广泛。当卡铂长期大量给药时会导致患者发生以血小板减少为主的骨髓抑制，影响正常造血与肿瘤治疗进程。微载体为一种新型自主研发 3D 仿生材料，模拟骨髓龛结构，能够为造血细胞提供物理环境支持与保护作用。我们通过构建卡铂致小鼠血小板减少模型并在小鼠骨髓腔内给予微载体，通过尾静脉取血检测血常规计数，在血小板降低程度最低时通过流式细胞术检测小鼠 HSC 及各系分化比例变化发现，微载体能够在小鼠生存、血小板抑制程度等方面表现出较好的骨髓保护作用。这些结果表明微载体能够降低卡铂所致的血小板减少相关的不良反应，也提示了微载体在抗肿瘤药物所致的骨髓抑制保护作用的潜在应用，为临床提供一种新型预防手段，为患者治疗提供更广阔的治疗窗。

【关键词】 卡铂 血小板减少 微载体

细胞竞争是阻碍跨物种嵌合体形成的壁垒之一

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【摘要】受到不同物种间进化差异和发育障碍的壁垒限制，导致人诱导多能干细胞在其他物种胚胎内嵌合难度大，总体嵌合比例较低。利用多能干细胞共培养模型，发现不同物种间的干细胞在特定阶段普遍存在由于细胞竞争力差异导致细胞竞争现象的发生，而且这种竞争力状态似乎跟物种间进化距离密切相关。在种间嵌合体形成过程中，供体的细胞可能被视为异常的细胞而通过细胞竞争被消除，而克服种间干细胞竞争可以提高供体多能干细胞在异种嵌合体里的嵌合度。因此，在进化距离更远的物种之间无法实现高效率的嵌合。

【关键词】 异种嵌合体；多能干细胞；细胞竞争

Hematopoietic Support and Immune Regulation Roles of Microcarrier in Immune-mediated Bone Marrow Failure

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【**Abstract**】 Aplastic anemia (AA) is a kind of bone marrow (BM) failure disease characterized by pancytopenia with decreased platelets, white cells and erythrocytes, and marrow hematopoietic failure.Severe thrombocytopenia may require platelet transfusion for the management and prevention of bleeding. In most patients, this is due to an immune attack by auto-reactive T cells against hematopoietic stem cells (HSCs). Therefore, AA is very instructive and provides insights into the quantity and regenerative ability of normal HSCs and autoimmune regulatory mechanism. In this study, we tested efficacy of microcarrier as a new or alternative treatment in bone marrow cell samples from clinical patients and mouse models of immune-mediated bone marrow failure. Our results demonstrated that the proportion and absolute numbers of hematopoietic stem and progenitor cells were increased when BM mononuclear cells derived from AA patients were cultured in vitro with microcarrier. Further study found that intra-BM injection of microcarrier improved peripheral platelet levels, ameliorated BM pancytopenia, and extended animal survival. Mechanistically, we speculated that microcarrier effectively preserved megacaryocyte (MK)-biased HSCs, which may give rise to immune MKs and stimulate expansion of functional regulatory T cells to downregulate the inflammatory process, and promote traditional MKs to increase platelet production. Taken together, these findings suggest that microcarrier play a role in maintaining HSCs and modulating autoimmune disorder, and it could serve as a promising complementary treatment of AA.

【**Keywords**】 Microcarrier, Hematopoietic Stem Cells, Aplastic Anemia, Bone Marrow Failure Disorders

The therapeutic effect of stem cells from human exfoliated deciduous teeth transplantation on a rat model of respiratory fistula

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【**Abstract**】Background: This study explored the therapeutic effects of stem cells from human exfoliated deciduous teeth (SHED) in respiratory fistula (RF) models in rats. Methods: RF models have been established with SD rats using a 1.5mm surgical punch. The animals were randomly divided into five groups: the normal group, the RF+ phosphate-buffered saline (PBS) locally group, the RF+ PBS intravenously group, the RF+SHED locally group, and the RF+SHED intravenously group. At two weeks, non-invasive testing was measured to evaluate lung function. Some rats were sacrificed one week after transplantation, the fistula repair was observed using the stereomicroscope, lung inflammation and histopathology was evaluated using HE, and immunofluorescence was performed to evaluate the survival of the transplanted SHED. At the end of the experiment, immunohistochemistry and western blot were used to assess the expression levels of the Toll-like receptor 4 (TLR4) and the Nucleotide-binding Oligomerization Domain-like Receptor Family Pyrin Domain Containing 3 (NLRP3) signaling pathways. Results: The fistula repair was better, and lung inflammation was obviously reduced in the RF+SHED group. No significant difference in pulmonary function was observed after transplantation. Our findings also showed that SHED was detected near the fistula and in the lungs in the RF+SHED group, and the majority of the intravenously injected SHED accumulated in the lungs after one week. In addition, TLR4 and NLRP3 expression were lower in the treatment group and SHED transplantation reduced RF-induced TLR4 and NLRP3 signaling pathways reactivity. Conclusion: The use of SHED to treat respiratory fistula has shown significant restorations in rat models. These findings provide additional evidence that SHED may be used in the cellular treatment of respiratory fistula.

【**Keywords**】 stem cells from human exfoliated deciduous teeth; respiratory fistula; cell therapy; TLR4; NLRP3

3D 仿生微载体重建急性移植物抗宿主病小鼠免疫与造血的机制研究 Immune and hematopoietic mechanism of acute graft-versus-host disease mice reconstructed by 3D biomimetic microcarrier

Hui Xu、Mei He、Ming Yang、Yinghui Li、Chaoqun Wang、Hexiao Zhang、Yiran Zhang、Yingdai Gao
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【**Abstract**】Background: Acute graft-versus-host disease (GVHD) , namely one disease caused by donor-derived $CD4^+$ T cells recognizing recipient cells as foreign antigen and then attacking them, is a detrimental by-product of allogeneic hematopoietic stem cell transplantation (HSCT). Both implementation of pre-HSCT conditioning regiments and occurrence of aGVHD disrupt the microenvironment of thymus except for gastrointestinal tract, skin, and liver, resulting in damaged thymopoiesis and decreased thymic export. There are two pathways for immunology reconstruction in recipient: one is expansion of donor-derived mature T cells in peripheral circulation, and the second is proliferation of donor stem cell-derived T cells in thymus. Damaged thymus make donor stem cell-derived T cells cannot fully be tolerized in recipient thymus and then auto-reactive $CD4^+$ T cells escape into the circulation, increasing the risk of infection，aggravating the level of aGVHD and transfering into chronic GVHD. Additionally, donor stem cell-derived common lypholoid progenitor (CLP) was lower in recipient peripheral circulation and bone marrow, causing defects in producing mature B cells.

Although having been extensively tested, a recognized, optimal standard strategy to prevent or treat aGVHD is still in exploration, due to high risk of infection and short survival. Unlike any of previous strategies, such as complete or partial T cell deletion, induction of donor T cell, or administration of anti-T cell antibodies, the novel material, microcarrier, may provide new insights into the approach to solve the major obstacle of HSCT--aGVHD.

Methods: In this study, to confirm the expansion potential of microcarrier, $CD34^+$ cells sorted from human umbilical cord blood (UCB) by magnetic activated cell sorting (MACS) were cultured *ex vivo* for 7 days and then cultured cells were sorted for RNA-seq analysis. To research the relationships between hematopoietic reconstruction promoted by microcarrier and immune reconstruction post transplanting in aGVHD, an aGVHD model with haplo-MHC-matched murine bone marrow transplantation was built after microcarrier (25μL/mouse, 25mg/mL) or equivalent solvent PBS being administrated via intramedullary injection.

Results: *Ex vivo* culture experiment showed that Microcarrier could expand $CD34^+$ cells sorted from human UCB and primarily expand immune progenitors and downstream mature immune cells，among which $CD4^+$ plasmacyloid DCs were significantly expanded through the analytic result of RNA-seq. In terms of survival, the group of aGVHD with microcarrier could outlive the group of aGVHD with PBS by nearly two weeks. From the

aspect of typical symptoms of aGVHD, aGVHD-micro group was significantly relieved compared to aGVHD-PBS group. In the following step, the frequencies and absolute numbers of each blood cell lineage in peripheral and bone marrow will be regularly monitored through flow cytometry to identify in which compartment microcarrier plays a role.

【**Keywords**】Graft-versus-host disease; Mouse model; RNA-Seq; 3D biomimetic material; Immune cells; Hematopoietic stem and progenitor cells; Flow cytometry

IFI16 promotes human embryonic stem cell trilineage specification through interaction with p53

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【**Abstract**】 Transcriptional regulation plays an essential role in the self-renewal and differentiation of human embryonic stem cells (hESCs). However, how external signals disrupt the self-renewal regulatory network and further drive hESC differentiation remains largely unknown. Here, we found the immune regulative protein, gamma-interferon-inducible protein 16 (IFI16) was involved in the regulation of both self-renewal and differentiation gene expression during hESC trilineage specification through interaction with p53. IFI16 expression levels were upregulated through JNK activation. IFI16 knockdown delayed the downregulation of self-renewal gene expression and suppressed the upregulation of differentiation gene expression, while IFI16 overexpression accelerated trilineage specification. Furthermore, IFI16 stabilized p53-binding in the genome through IFI16-p53 interaction and differentially regulated self-renewal and differentiation gene expression. Together, our results suggest a particular role of IFI16 in differential gene expression regulation during trilineage specification of hESCs in a manner that is dependent on the genome-wide profile of p53-binding directed by IFI16-p53 interaction.

【**Keywords**】 IFI16, p53, trilineage specification, human embryonic stem cells

Base Editing Mediated Generation of Point Mutations Into Human Pluripotent Stem Cells for Modeling Disease

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【**Abstract**】Human pluripotent stem cells (hPSCs) are a powerful platform for disease modeling and drug discovery. However, the introduction of known pathogenic mutations into hPSCs is a time-consuming and labor-intensive process. Base editing is a newly developed technology that enables facile introduction of point mutations into specific loci within the genome of living cells. Here, we design an all-in-one episomal vector that expresses a single guide RNA (sgRNA) with an adenine base editor (ABE) or a cytosine base editor (CBE). Both ABE and CBE can efficiently introduce mutations into cells, A-to-G and C-to-T, respectively. We introduce disease-specific mutations of long QT syndrome into hPSCs to model LQT1, LQT2, and LQT3. Electrophysiological analysis of hPSC-derived cardiomyocytes (hPSC-CMs) using multi-electrode arrays (MEAs) reveals that edited hPSC-CMs display significant increases in duration of the action potential. Finally, we introduce the novel Brugada syndrome-associated mutation into hPSCs, demonstrating that this mutation can cause abnormal electrophysiology. Our study demonstrates that episomal encoded base editors (epi-BEs) can efficiently generate mutation-specific disease hPSC models.

【**Keywords**】Brugada syndrome; IPS; base editing; disease modeling; episomal vector; human pluripotent stem cell; long QT syndrome

细胞骨架硬度对干细胞衰老的调控机制

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【摘要】细胞的衰老不仅是个生化过程，也伴随着深刻的物理机械性能的变化，尤其体现在细胞骨架的特性。细胞骨架在衰老相关疾病中的变化和作用被陆续报导，然而细胞骨架机械性能对细胞衰老的主要影响方式和调控机制尚未被了解。我们的研究旨在揭示细胞骨架机械性能在介导干细胞的加速衰老进程中的潜在作用。 Hutchinson-Gilford Progeria Syndrome (HGPS) 哈-格二氏早老综合症，是因为 Lamin A (LMNA) 基因的突变造成的致死性过早衰老疾病，现有技术无法治愈。HGPS 细胞中 LMNA 基因的突变造成早老蛋白（早老素）Progerin 的表达产生，继而代替 Lamin A 在核纤层（nuclear lamina）中大量聚集和核纤层硬度的明显升高。然而，HGPS 细胞中 Progerin 的表达导致的细胞核机械性能的变化 对细胞衰老的介导机制尚未被了解。我们对 HGPS 病人皮肤干细胞的研究发现，核纤层硬度的增加会导致细胞骨架的硬度明显增加，两者相互作用导致细胞核结构的异常（即核出泡或微核形成）。而细胞核异常可导致细胞质内携带 DNA 的微核(micronuclei)的增多，从而激活 cGAS-Sting 等天然免疫信号和诱导加速细胞衰老。我们对 HGPS 的小鼠模型（Zmpste24-/-）的肌肉干细胞的研究也发现，衰老的肌肉干细胞具有更高的细胞骨架密度和硬度，这和细胞核形态的异常畸变紧密相关。因此，我们的研究提高了对细胞机械特性在介导干细胞衰老及相关疾病中的作用机制的了解。

【关键词】 干细胞衰老，细胞骨架，天然免疫，早老素

高活性肌肉干细胞及外泌体对盆底肌损伤的修复

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【摘要】盆底功能障碍性疾病(pelvic floor dysfunction, PFD)是影响女性生活质量的常见病，包括压力性尿失禁(stress urinary incontinence, SUI)、盆腔脏器脱垂和性功能障碍等,发病率可高达 20%~40%。而受生育、疾病、衰老等因素的影响，30 岁以上的女性盆底功能障碍性疾病的发生率高达 40%～50%。女性的盆底主要是由肌肉和筋膜组成的，而盆底功能障碍性疾病一般与盆底肌的损伤直接相关。现阶段治疗盆底肌损伤相关尿失禁的主要方式为药物治疗、盆底康复治疗和手术治疗等，然而这些方法的治疗效果并不理想。理论上最直接有效的治疗方法是激活盆底肌的修复和再生。近十年来，肌肉干细胞注射对盆底肌损伤相关尿失禁的治疗应用得到重视和发展，而相关临床试验和小范围的初步实用也证实了肌肉干细胞对尿失禁病人的积极正面作用。然而，不仅肌肉干细胞本身，肌肉干细胞分泌的外泌体囊泡也被证明有强大的帮助肌肉修复的功能。我们的近期研究也证明了肌肉干细胞外泌体囊泡的高效的抗氧化抗炎症功能。此项研究旨在验证利用高活性的肌肉干细胞外泌体，通过在受损伤盆底肌肌肉中的直接注射来建立有效安全治疗改善盆底功能障碍的方法。我们的实验结果表明，在年老小鼠盆底肌损伤后，对照组小鼠盆底肌修复缓慢并且产生大量纤维化组织，而连续 10 天的外泌体的注射可以有效加快盆底肌的修复而且减少纤维化的产生。而且外泌体注射可以有效减少衰老细胞的数量并抑制细胞内促衰老基因的表达。因此，高活性肌肉干细胞外泌体具有被运用来治疗盆底肌损伤相关疾病的可能。

【关键词】 外泌体，肌肉干细胞，盆底肌，尿失禁

Human platelet lysate (hPL) alters the lineage commitment and paracrine functions of human mesenchymal stem cells via mitochondrial metabolism

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【 **Abstract** 】 Emerging evidence indicates that cellular bioenergetics is critical in determining stem cell self-renewal and differentiation. Human platelet lysate (hPL) has showed the capability to improve mesenchymal stem cell (MSC) self-renewal and differentiation. However, the detailed modulating effect of hPL on MSCs energy metabolism remain unexplored. This study investigated the effect of different concentrations of hPL on human MSCs. The results showed that the cell size and cell spreading area were decreased, but the cell proliferation and osteogenesis of MSCs were improved in hPL compared to the fetal bovine serum (FBS) control. 5% hPL exhibited a better matrix mineralization effect than 10% FBS on MSCs from different origins. RNA sequencing results revealed widespread transcriptome differences between hPL and FBS- cultured MSCs (hPL-MSCs and FBS-MSCs) where the different expressed genes (DEGs) were enriched mainly in metabolic and PI3K-Akt signaling pathway. The most important finding was that the PI3K-Akt/HIF1A-mediated metabolic state dominated the physiological property and lineage commitment of MSCs in hPL. In response to the down regulated HIF1A (hypoxia inducible factor 1 alpha) of hPL-MSCs, energy metabolism switched from glycolysis towards mitochondrial oxidative phosphorylation (Oxphos) featured by an elongated mitochondrial network and reduced mitochondrial membrane potential. In hPL, MSCs tend to differentiate towards the aerobic metabolism-demanded osteocytes and adipocytes rather than the anaerobic-chondrocytes and the immunomodulatory and angiogenic capacity of MSCs were impaired. This study unveils the modulating mechanism of hPL on MSC lineage specification and paracrine function, which could give instructions for both MSC and hPL-based application in regenerative medicine.

【 **Keywords** 】 human platelet lysate, mesenchymal stem cell, lineage commitment, mitochondrial metabolism, paracrine functions

基于生物 3D 打印的体外肿瘤模型及其在个性化治疗中的应用研究

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【摘要】我国目前正面临着严峻的肿瘤防治形势。根据国家癌症中心 18 年的数据，全国新发病例数超过 380 万例，死亡数达到 230 万例。发病率和死亡率都高于全球平均值，发病人数全球第一。遗憾的是，目前尚无一种彻底治愈肿瘤的治疗方法，这也与肿瘤组织的复杂性密切相关。个性化医疗是 2015 年以来被提出的治疗概念，该方法也被称为“癌症精准医疗”，是从癌症的筛查、评价，有效药物或治疗方案的选择到临床前检验相结合的一体化方案。这种方法需要整合病人的病理学、基因组学、蛋白组学、药物基因组学、免疫基因组学信息来进行个性化抗癌治疗、检测复发、优化治疗效果、最小化病人的边际效应、获得最佳的治疗效果。我们的工作则是基于细胞 3D 打印技术在仿生结构设计、多材料组分组装等方面的优势，将肿瘤细胞和生物材料作为原料，按细胞特定微环境要求，制造用于个性化药物筛选的临床前体外肿瘤模型。具体包括：

1）参与设计并开发了可实现单细胞打印精度的集成 3D 打印系统及设备，实现多种细胞/材料在几十微米内的精确定位，成功构建了体外三维异质肿瘤模型。并通过单个细胞打印操控，构建了单细胞水平的“肿瘤-免疫相互作用模型”，在体外捕捉到单个 T 细胞对肿瘤细胞的杀伤过程；

2）首次以 3D 打印构建了患者来源的肝癌及肝内胆管癌模型，模型内肿瘤细胞与亲代肿瘤细胞的基因表达在转录组水平高度一致，药效学评价接近体内结果，可望成为精准医疗研究的有力手段；

3）对体外肿瘤模型中癌细胞经生长转化因子诱导的上皮-间质转化进行了系统研究，追踪获得癌细胞的迁移行为，提供了一种更好的上皮-间质转化理解机制，有助于更好地了解宫颈癌转移规律，可用于未来临床治疗方案制定。

【关键词】 生物 3D 打印；个性化肿瘤模型；肿瘤-免疫相互作用；上皮-间质转化；精准医疗

Explore the Role of the Antioxidant Medium in Improving the Efficiency of hPGCLCs Induction

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【**Abstract**】The primordial germ cells (PGCs) are the founding population of the germ-cell lineage which eventually differentiate into either oocytes or spermatozoa depending on the sex of the individuals and it has a relatively high level of pluripotency.

In recent years, there have been several methods successfully adopted in inducing human primordial germ cell-like cells (hPGCLCs) in vitro. With consistent cytokines' usage, though, the induction efficiency varies among different basic media including GK15 and arB27.

We now find a new induction system using DF12 as basic medium, with B27 and N2 as supplements, consistent with four cytokines: BMP4, SCF, EGF and LIF. PGCLC induced is close to PGC from 7W embryo at the transcriptome level. N2B27 makes a much higher speed and efficiency of induction if compared with traditional GK15. Through RNA-seq, we found that the advantage comes from how cells deal with hypoxic stress and apoptosis. N2B27 medium made cells a weaker generation of reactive oxygen species (ROS) and a higher mitochondrial activity to better deal with hypoxic stress. Furthermore, by analyzing the composition of GK15 and N2B27, we found that antioxidants, which is much richer in N2B27, may play a role through the induction process. The optimal concentration addition of N-acetyl-L-cysteine (NAC), a common antioxidants, leads to some extent the increase of PGCLC induction efficiency in GK15 observed by FACS and IF after a gradient concentration experiment.

In conclusion, the N2B27 induction system we pioneered brings obvious advantages to hPGCLC induction, in which antioxidants have a positive effect.

【**Keywords**】hPGCLC、GK15、N2B27、N-acetyl-L-cysteine (NAC)

The functional heterogeneity of adult hippocampal neurogenesis along the dorsal-ventral axis

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【**Abstract**】In the mammalian hippocampus, there are still a large number of neural stem cells during adulthood, and new neurons continuously arise from stem cell through the processes of proliferation, differentiation, development and integration, known as adult hippocampal neurogenesis (AHN). These adult-generated neurons have higher sensitivity and plasticity, and may play a key role in learning and memory, anxiety and stress regulation, and social behavior. Adult neurogenesis has been proven to be impaired in hippocampus of patients with mood disorders and neurodegenerative diseases. AHN is an interesting target to better understand dysregulation of mood and memory, and might be a converging mechanism for behavioral dysfunction. However, evidence supporting a role for AHN in neurological and psychiatric disorders has remained sparse and inconclusive. It is far from clear how modulating adult neurogenesis affects neuropathology. There are controversial results in the data from experimental animals targeting AHN. We hypothesize that the controversy may be due to the lack of specificity in regulation techniques. The complexity and heterogeneity of AHN have been ignored. In the present study, we explore the functional heterogeneity of adult hippocampal neurogenesis along the dorsal-ventral axis. By adjusting the dorsal and ventral AHN separately, we show that the dorsal AHN is involved in the learning and memory of spatial and object cues, and the ventral AHN is involved in regulating innate anxiety and avoidance behavior. Social behavior and social memory require the participation of both. Selective ablation of dorsal AHN causes a decrease in learning speed of spatial cues, decline in recognition of new objects, impaired memory retention, and reversal learning ability. Ablation of ventral AHN show a significant reduction in innate anxiety and avoidance behavior in elevated plus maze and open field, also a shorter latency of feeding in novel environment. Regardless of ablation of dorsal or ventral AHN, it causes the impairment of social novelty memory. These evidences suggest that AHN is indeed directly involved in behavioral functions such as cognition, emotion, and social interaction. We conclude from these data that AHN is necessary for the survival of individuals and the maintenance of the population. It also challenges the previous perception that the lack of neural stem cells in adult individuals does not affect behavior and survival. Moreover, the function of AHN is separated along the dorsal-ventral axis. Such functional heterogeneity should be considered when targeting AHN in treatment of neurological diseases.

【**Keywords**】Adult hippocampal neurogenesis，Mood disorders，Cognitive impairment，Neuroinflammation，Neural stem cell

Repeated peripheral LPS challenges induce prolonged neuroinflammation through astrocytic-microglial complement C3a cascade

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【**Abstract**】Background: Neuroinflammation in neurodegenerative diseases is usually chronic and unresolved, but how this type of neuroinflammation is established remains unclear. Recently, it has been shown that the activation of complement C3a-C3aR drives local inflammatory tissues priming and causes prolonged arthritis. Meanwhile, the expression of C3 and C3aR are increased in neurodegenerative diseases such as AD, PD and ALS. However, whether complement C3a-C3aR activation contributes to the prolonged neuroinflammation is unknown.

Methods: Lipopolysaccharide (LPS) was administrated to mice once or twice to induce neuroinflammation. Neuroinflammation is assessed by examining the expression of pro-inflammatory cytokines in brain tissue. Cellular localization of C3 and C3aR was inspected by immunofluorescence staining. To block C3a-C3aR interaction, C3aR antagonist was injected intraperitoneally. Effects of C3 and C3aR agonist were also essayed in vitro using BV2 microglia cell line.

Results: Upon LPS administration, intense neuroinflammation was induced within 3 hours, but gradually faded 24 hours later. With an interval of 7 days between two challenges, LPS induced reduced but prolonged neuroinflammation accompanying increased C3 expression. The increased C3 was mostly derived from astrocytes, while C3aR was expressed on microglia. Then, the complement C3a receptor antagonist SB290157 was injected intraperitoneally into mice that were repeatedly challenged with LPS and we found that prolonged neuroinflammation was abolished. In vitro, complement C3 or C3aR agonist enhanced NLRP3 inflammasomes activation in LPS-treated microglia.

Conclusion: Our results indicate that compared with a single LPS, two LPS performed at 7-day intervals can induce reduced but prolonged neuroinflammation. Blocking C3a receptors blocks this prolongation. We provide a new perspective on the role of the complement system in the prolongation of neuroinflammation.

【**Keywords**】neuroinflammation, C3, C3aR, astrocytes and microglia.

CONSTRUCTION OF PANCREATIC ISLET-LIVER MULTI-ORGANOID-ON-CHIP SYSTEM FROM HIPSCS

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【**Abstract**】The cross-talk between pancreatic islet and liver plays an important role in glucose regulation. The dysfunction of the interplay between islet and liver will result in metabolic disease, such as type 2 diabetes mellitus (T2DM). Multi-organ-on-chip systems provide more in vivo-like models to emulate human biology, which enables detailed study of the interplay between two or more organs. Recently, organoids derived from human induced-pluripotent stem cells (hiPSCs) became a robust platform for disease molding and drug testing. Here, we propose a new hiPSC-derived multi-organoid-on-chip (MOC) system that allows to recapitulate the interplay between islet and liver in glucose regulation. The established robust islet and liver MOC system provides a promising platform for glucose metabolism,T2DM study and drug screening.

KEYWORDS: Islet, Liver, Multi-organoid-on-chip, HiPSCs

INTRODUCTION

In vivo, glucose regulation accomplished by complex and dynamic organs' cross-talk, such as liver and pancreatic islet. However, the in vitro models to study the cross-talk between human organs for disease pathophysiology and therapeutic drug evaluation is lacking. Multi-organ-on-chip systems are recognized as promising approach to co-culture various organs or organoids for studying the organ cross-talk in normal and disease status. [1].Organoids generated via self-organizing of pluripotent stem cells (PSCs) in vitro presents a new class of three-dimensional tissues, which can reflect the organ-specific structures and functions of their native counterparts [2]. Organoids-on-chip system combines the advantages of microengineering technique and stem cell-derived organoids, representing a novel approach for modeling the interplay between liver and islet. Here, we proposed a new strategy for co-culture of the pancreatic islet and liver organoids derived from hiPSCs using a MOC system to recapitulating their interplay in glucose regulation.

RESULTS AND DISCUSSION

In this work, we present a new strategy for co-culture of liver and islet in a MOC system that enables long-term culture of organoids with favourable cell viability. We assess the insulin and albumin (ALB) secretions in islet and liver organoids, the results demonstrated that, compared to organoids in mono-culture condition, the organoids in co-culture system secreted more insulin and ALB, indicating the co-culture condition could enhance the function of islet and liver organoids. Besides, the expressions of organ-specific proteins in islet and liver organoids were increased in co-culture system, confirming the effects of organs' interactions on tissue maturity. Overall, all above results indicated that the co-culture system facilitated the maturation and secretion function of liver and pancreatic islet organoids in the pro-longed culture period.

CONCLUSION

In summary, we recapitulated the cross-talk between liver and islet in vitro using the microfluidic technology combined with hiPSCs derived organoids. The biomimetic multi-organoid chip system displayed the organ-specific structures and functions of liver and islet, which holds the potential for normal regulation of glucose levels. In addition, the islet and liver MOC system provide a promising platform for disease studies and drug testing.

ACKNOWLEDGEMENTS

This research was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences, Grant (No. XDA16020900, XDB32030200).

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【**Keywords**】Islet, Liver, Multi-organoid-on-chip, HiPSCs

小鼠雄性生殖细胞在全局发育过程中的细胞命运转变和测定分析

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【**Abstract**】The mammalian male germ cell development is initiated from specified PGCs to sperms after stepwise cell-fate transitions; however, the full-term developmental profile of male germ cells has not been defined. Here, by interrogating the high-precision transcriptome atlas of 11,598 individual cells covering 28 critical time-points throughout mouse germline development, we established an unbiased male germ cell developmental landscape, which demonstrated that cell-fate transition from mitotic to post-mitotic PGCs was driven by whole transcriptome-scale reconfiguration and a newly defined transitional cell cycle progression. Importantly, the Notch signaling pathway was validated to be essential for initiating mitotic arrest and the maintenance of male germ cells' identities. Furthermore, we also proved that ablation of a previously reported male subfertility associated gene HELQ could induce aberrant progression of transitional PGCs and developmental arrest of post-mitotic PGC stage as well as abnormal transcriptome reprogramming, further indicating the importance of cell cycle regulation for proper cell-fate transition in late-stage PGCs. Finally, systematic human-mouse comparison of germ cell development revealed a core set of potential regulators whose deficiency contributed to human male infertility via mitotic arrest regulation. Collectively, our study provides an accurate and comprehensive transcriptome atlas of the male germline cycle and allows for an in-depth understanding of the cell-fate transition and determination underlying male germ cell development.

【**Keywords**】Male germ cell developmental landscape；Cell-fate transition；Mitotic to mitotic arrest PGC transition；Notch signaling pathway；Helq；Human-mouse comparison dataset

AKT3 de novo mutation in hemimegalencephaly induces cooperative disorders of the adjacent nonmutant cells

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【**Abstract**】De novo mutation in brain cells of hemimegalencephaly accounts for the abnormal expansion of cerebral hemisphere and cortical dysplasia. Yet how very few mutant cells cause significant hemisphere enlargement remains unclear. *AKT3* mutated hESCs were used to form organoids and simulate the mosaic in HME patients. Mutation itself significantly promoted the cell proliferation of NPCs and increased the size of organoids. Mosaic *AKT3* model induces a similar alteration in both the mutant and nonmutant cells, thereby accumulated a large number of neurons that were abnormal in structure and function. In between normal and mutant cells, there are increased number of shortened cilia accompanying excessive N-cadherin accumulation. These changes trapped the cells within the lumen side in VZ. Thus, targeting N-cadherin or ciliogenesis may be beneficial to the deteriorating cortical dysplasia, which promises a valuable intervention for such somatic mutation diseases.

【**Keywords**】AKT3，hemimegaloencephaly, cerebral organoids, N-cadherin, primary cilia

Efficient production and image-based evaluation of retinal pigment epithelial sheets from human pluripotent stem cells

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【**Abstract**】 Purpose Transplantation of human pluripotent stem cells (hPSCs) derived retinal pigment epithelial (RPE) sheets is a promising therapy for retinal degenerative diseases such as AMD and RP. However, this regenerative therapy faces challenges of the large-scale production and quality control of RPE sheets. This study aimed to develop a highly efficient and reproducible differentiation protocol by using small molecules to produce RPE cells with hPSCs in a large scale, and construct RPE sheets for cell transplantation of retinal diseases. In addition, image-based quality evaluation was also developed to predict the function of RPE sheets, facilitating the RPE transplantation.

Methods and results We developed an optimized dual-Smad inhibition combining with other 4 small molecules (RPE6iN protocol) to induce hPSCs into RPE cells in high efficiency. After 12 days of inducing, hPSCs differentiated into RPE progenitors, which were double-positive for MITF/PAX6. We found that the treatment of nicotinamide further up-regulated the efficiency of RPE cells production because nicotinamide induced cell apoptosis in non-RPE cells. The polygonal morphology of pigmented cells was identified at day 35, which is the typical morphological feature of RPE cells. Cell purity was accessed before and after cell passage by immunostaining. Over 99% of cells were identified as RPE cells after cell passage. At least 5 different hPSC lines were proved to generate RPE cells in high efficiency by this protocol. After seeding into transwell, RPE cells became polarized mature epithelia with heavy pigmentation, and expressed mature RPE markers four weeks after culture. These RPE sheets also had the potential of phagocytosis of POS and barrier function.

To develop a functional prediction for RPE sheets, F-actin labeled images of RPE sheets were used to construct a machine learning model. The machine learning prediction model was able to eliminate the RPE sheets with poor barrier function (accuracy: 99%; sensitivity: 99%; specificity: 100%). When we adapted the prediction model to recognize the morphological features from phase contract images of RPE sheets, the performance of this model was practically effective (accuracy: 88%; sensitivity: 88%; specificity: 88%).

Conclusion Here we developed a novel protocol RPE6iN to efficiently induce differentiation of hPSCs into RPE cells, enabling the preparation of high-purity RPE cells and mature RPE sheets without specific manual selection. We also constructed a non-invasive, machine learning-based TER prediction model to discriminate RPE sheets with bad barrier function. These achievements would significantly promote the basic and translational studies of hPSCs-derived RPE cells.

【**Keywords**】 Cell therapy; Pluripotent stem cell; Retinal pigment epithelium; RPE replacement therapy; Tissue engineering; Machine learning

VSX2 荧光报告视网膜类器官活体动态揭示人视网膜细胞命运转变及转录调控

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【摘要】目的：视网膜是中枢神经系统的外延部分，是神经元细胞生理与病理研究的经典模型。由于人类视网膜组织来源有限且无法直接进行分子操作，人类视网膜发育和疾病的调控机制还很不清楚。VSX2 是调控视网膜发育的关键转录因子。本研究旨在构建 VSX2 绿色荧光报告（VSX2-eGFP）人诱导多能干细胞（hiPSCs），获取视网膜类器官，动态监测人视网膜发育进程，细胞命运转变及转录调控。

方法：根据 CRISPR/Cas9 技术原理构建 VSX2-eGFP hiPSC 系，并进行特性鉴定。通过我们已报道方法进行 hiPSCs 的诱导分化获得三维视网膜类器官（ROs）。通过倒置荧光显微镜活体追踪 VSX2-eGFP 在视网膜分化过程中的动态表达。免疫荧光，流式细胞分选技术和 RNA-seq 评价 VSX2-eGFP+ 细胞命运转归及转录组。

结果：VSX2-eGFP hiPSCs 维持了亲本的特征，并分化获得了 ROs，包括所有视网膜神经元和视网膜色素上皮细胞（RPEs）。VSX2-eGFP 模拟了 VSX2 在体内的表达模式，只表达在神经视网膜（NR），而不表达在 RPEs。在分化早期，VSX2-eGFP 定位于视网膜前体细胞（RPCs），晚期定位于双极细胞（BCs）。RNA-seq 分析表明，随着培养时间的增加，通过调控一系列转录因子及基因的表达，VSX2-eGFP+ 细胞的命运实现了从 RPCs 向 BCs 的转变。最后，利用 RNA-seq 分析结果，我们获得了早期 RPCs 和晚期 BCs 潜在的表面标记物。

结论：成功构建了 VSX2-eGFP hiPSC 系，并分化获得报告 ROs；动态监测到人视网膜的发育进程，揭示了 VSX2-eGFP+ 细胞命运转变；建立了 VSX2-eGFP+ 细胞的动态转录组数据库，并提取了视网膜细胞潜在的表面标记物，为人视网膜发育及疾病、视网膜细胞替代疗法等研究奠定了基础。

【关键词】 CRISPR/Cas9；VSX2；荧光报告细胞系；hiPSC；视网膜类器官；人视网膜发育；细胞命运；转录组

Comparison of antiobesity effects of adipose-derived stromal/stem cells from different sources in a natural aging model

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【**Abstract**】Purpose: Our previous study found that white adipose stem cells (W-ASCs) derived from abdominal and femoral sulcus white adipose stem cells (ASCs) have antiaging and age-related obesity effects. Whether interscapular brown adipose stem cells (B-ASCs) have the same effect has not been reported. The study objective was to compare the effects of ASCs from different tissues on aging and aging-related obesity.

Patients and methods: C57BL/6J mice were transplanted with either B-ASCs or W-ASCs at 2 months of age. Changes in body weight, biochemistry, cytokines, hormone secretion, cell senescence, lipid metabolism, and ASC function in were assessed at 22 months of age.

Results: W-ASCs were superior to B-ASCs as aging and age-related obesity indicators, based on change in body weight, organ weight, antioxidant and anti-inflammatory activity, lipid metabolism, and liver and kidney function.

Conclusion: Difference in the tissue source was reflected by the heterogeneity of antiaging and age-related obesity effects of transplanted ASCs. Based on the study results, we recommend W-ASCs over B-ASCs in aging and age-related obesity applications.

【**Keywords**】adipose-derived stromal/stem cells; different tissue sources; obesity; aging

利用羊膜作为支架构建工程化视网膜色素上皮细胞片

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【摘要】目的: 人多能干细胞来源的视网膜色素上皮(RPE)细胞可为致盲性视网膜退行性疾病的细胞治疗提供无限细胞来源。然而，RPE 细胞悬液移植以非极化的方式递送细胞至视网膜下腔，细胞有上皮间质转化的风险，导致细胞治疗失败。本研究旨在构建工程化视网膜色素上皮片， 并评价其生物学特性，为 RPE 替代治疗提供移植材料。

方法: 采用由本实验室通过视网膜类器官分化方法获得 hiPSC-RPE 细胞(iRPE)，将其接种于去上皮的羊膜（hAM）支架上。普通平板和 transwell 用于对照培养 iRPE。采用倒置荧光显微镜、扫描电镜和透射电镜观察培养后 iRPE 细胞的形态特征;免疫荧光染色和 RNA-Seq 分析 RPE 特异性关键标记的表达;同时进行吞噬和分泌功能测定。

结果: 倒置显微镜及电镜观察，羊膜支架上培养的 iRPE 片（hAM-iRPE）细胞呈排列紧密的六边形，富含色素; 相比于普通平板和 transwell 培养的 iRPE，在羊膜支架上的 iRPE 细胞发生间质转化的梭形细胞数明显减少。 hAM-iRPE 表达特异性标记物 CRALBP、ZO-1、BestI、EZRIN、ATPase; 具有分泌和吞噬功能。RNA-seq 分析表明，hAM-iRPE 间质细胞相关标签明显下调，提示 hAM 具有抑制 iRPE 上皮间质转化(epithelial mesenchymal transition, EMT)。

总结：脱细胞羊膜可模拟天然 RPE 的基底膜，促进 iRPE 生长，并保持其生物功能。所获得的 hAM-RPE 薄片为视网膜疾病的细胞替代治疗提供了有价值的生物材料。建立的新型 hAM-RPE 培养系统可用于视网膜疾病建模和药物筛选。

【关键词】 视网膜色素上皮细胞; 羊膜支架; 组织工程;干细胞疗法;视网膜疾病

Different roles of polydopamine coating in controlling cell behaviors of MSCs and iPSCs

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【**Abstract**】Our previous studies demonstrated that the artificial matrix, binary colloidal crystals (BCCs), had significant impacts on cell morphology and lineage specification of stem cells. Herein, we further modified BCC with polydopamine (PDA), a cell-adhesive polymer, and studied the combined effect of BCC and PDA on the human adipose-derived stem cells (hASCs) and induced pluripotent stem cells (hiPSCs). The BCCs substrate was fabricated from the self-assembly of two colloidal suspensions (Si: 5μm and PS: 0.4μm). Several controls, including PDMS-embedded BCCs (BCCP), BCC structure replicates using polystyrene (BCCR), and PDMS imprinting of iPSCs, were prepared for the combined comparison. Assessments were carried out in the conditions of with PDA vs without PDA coating for chondrogenic differentiation of hASCs; and low vs high concentration of PDA for iPSCs culture. The substrates were additionally treated with gelatin and gelatin-Matrigel for hASCs and iPSCs cultures, respectively. Chondrogenic induction was done for 21 days and then our observation indicates that, while PDA coating prevents cell aggregation on all the studied substrates, it never appeared on the BCCs based substrates without coating; Consequently, BCCs and TCP with PDA coating had the highest amount of glycosaminoglycan (GAG) formation. On the other hand, the substrates with a higher amount of PDA induced iPSCs to form dome-like morphology while it arose with a lower concentration of PDA on the BCCs substrate. We believe that these two opposite results of cell collective behavior are linked to the differences in needs of two different types of stem cells (multipotent vs pluripotent). Extracellular matrix (ECM) bioactivity including motility seems to be a critical factor for iPSCs spreading that is hampered by a high concentration of PDA immobilization and BCCs; Our study shed the light on the importance of the substrates' physico-chemical features in the cells' collective behavior of two different types of stem cells which can further translate to various applications such as chondrocyte differentiation in a 2D culture system with a high cell density of mesenchymal stem cells and embryoid body formation from pluripotent stem cells through surface chemical functionalization.

【**Keywords**】Niche, Cartilage, Artificial matrix, Topography, Mesenchymal stem cells, iPSCs

Lepr+细胞在肾脏中的鉴定及其在肾纤维化过程中的生理学功能研究

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【摘要】间充质干细胞（Mesenchymal stem cell, MSC）是一类在成体组织中广泛存在的干细胞。骨髓中的 MSC 是成体骨骼和软骨的前体细胞，内脏组织中的 MSC 则是组织纤维化过程中新生成的成肌纤维细胞（Myofibroblast）的前体细胞。瘦素（Leptin）是一种主要由白色脂肪细胞分泌的与糖尿病密切相关的蛋白质类分泌激素。传统观点认为，Leptin 通过作用于海马体，使大脑分泌神经内分泌因子，从而间接调节机体各个组织的生理活动。周波老师的前期工作发现，瘦素受体（Leptin receptor, Lepr）是骨髓间充质干细胞的特异性标记物，从骨髓 MSC 中特异性敲除 Lepr 会导致骨质异常增生。这提示 Leptin 可以通过直接作用于 Lepr+ MSC 来调节骨骼发育。我们构建了新的 Lepr-creER 小鼠对 Lepr+ 细胞进行谱系示踪发现，Lepr 不仅表达于骨髓 MSC，也表达于多个髓外组织的 MSC 中，包括肾脏、心脏和肺脏等。根据以往的报道，Leptin 代谢与肾功能、糖尿病和肾纤维化都有着密切的联系，因此，我们建立了多种谱系示踪小鼠和条件性敲除小鼠品系，利用小鼠肾纤维化模型和糖尿病模型，证明肾脏中的 Lepr+ MSC 参与了急性或慢性的肾纤维化过程。同时，在肾脏中阻断 Leptin 信号通路会对疾病发展过程中纤维化的产生造成影响。这些工作将让人们对糖尿病和肾脏疾病的关系有新的认识，并为治疗此类疾病提供潜在的靶标。

【关键词】 肾纤维化，Leptin Receptor 通路，糖尿病

示踪发育过程中骨骼干细胞的转变

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【摘要】多种不同类型的骨骼干细胞已被证明参与软骨内成骨的发育和维持。然而，不同骨骼干细胞对骨骼发育分工的异质性以及他们之间的等级关系仍然不确定。因此，我们利用双同源重组酶谱系示踪系统来探究出生后骨形成期间的骨骼干细胞转变。我们发现，出生后的成骨细胞主要来自青春期前的软骨细胞和青春期后的 *Lepr*$^{+}$骨髓基质细胞(BMSCs)。这种转变发生在青春期的骨干部位，并逐步扩散到干骺端。*Lepr*$^{+}$骨髓基质细胞主要来源于胚胎软骨细胞。在围产期软骨细胞中和成体时期 *Lepr*$^{+}$ BMSCs 中条件性敲除 *Runx2* 分别影响骨的纵向伸长和横向增粗。小鼠运动实验表明长期奔跑有利于增强青春期前软骨细胞的成骨能力，但对成体时期的 *Lepr*$^{+}$ BMSCs 的成骨能力没有影响。因此，来源于早期软骨细胞的骨骼干细胞以及成体时期的骨髓基质细胞依次控制软骨内成骨的生长和维持。

【关键词】 骨骼干细胞，谱系示踪，Lepr

Efficacy of hUC-MSCs/TMZ combination in the treatment of glioblastoma

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【**Abstract**】 Mesenchymal stem cells have the ability to self-renew and multidirectionally differentiate, and the use of mesenchymal stem cells for cancer treatment is an emerging therapeutic approach. Glioblastoma is a highly devastating primary brain tumor that is resistant to conventional therapeutic agents. Both human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) and temozolomide（TMZ） have been shown to inhibit glioblastoma, but there is little information on the combination of hUC-MSCs/TMZ for the treatment of glioblastoma. We investigated the efficacy of human umbilical cord mesenchymal stem cell (hUC-MSCs) and TMZ combination therapy for glioblastoma (RG-2, U87-MG, and U251) and constructed an in situ rhodopsin model in nude mice to further evaluate the efficacy and feasibility of hUC-MSCs/TMZ combination therapy for tumor suppression in glioblastoma cells; and to explore the effects of hUC-MSCs/TMZ on MGMT expression regulation and the related mechanism of apoptosis and autophagy mediated by endoplasmic reticulum activation pathway. The results showed that the combined administration of hUC-MSCs/TMZ successfully detected different degrees of inhibition on the proliferation, invasion and migration of glioblastoma RG-2, U87-MG and U251 cells; and regulated the expression of MGMT and the related mechanisms of apoptosis and autophagy mediated by the endoplasmic reticulum activation pathway. We successfully constructed an in vivo tumor model in nude mice, and verified that UC-MSCs could kill U87-MG and U251 cells to different degrees in nude mice by in vivo assays.

【**Keywords**】 human umbilical cord-derived mesenchymal stem cells 、 Glioblastoma、Temozolomide、Autophagy、Apoptosis

Black Phosphorus Nanosheets Mediate the Differentiation of hiPSCs to Generate Neural Progenitor Cells with Protective Effects against Ischemic Stroke

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【**Abstract**】Stem cell transplantation is a promising therapeutic strategy for ischemic stroke. It is a reasonable strategy to enhance the antioxidant capacity of stem cells through nanomaterial treatment to improve the therapeutic effect of transplanted stem cells in the oxidative damage area. In this study, we used black phosphorus nanosheets (BPNs) to mediate differentiation of human induced pluripotent stem cells (hiPSCs) into neural progenitor cells (NPCs) with stronger antioxidant capacity. the hiPSCs-NPCs treated with BPNs could express more antioxidase, while showing better protection against hydrogen peroxide induced neuronal damage. In vivo experiments showed that compared with the PBS group, the NPCs transplanted groups could effectively alleviate neurological damage in stroke rats with middle cerebral artery occlusion (MCAO). Interestingly, compared with normal NSCs, NSCs pretreated with BPNs could more effectively inhibit lipid peroxidation and reduce oxidative damage and neuronal apoptosis in the brain tissue of stroke rats. It also inhibited inflammation and the glial scar by inhibiting the activation of astrocytes. Our study provides new insights for the application of BPNs in stem cell transplantation for the treatment of ischemic stroke.

【**Keywords**】 black phosphorus nanosheets, neural progenitor cells, ischemic stroke, neurological repair

Gelatin microspheres loaded with Wharton's jelly mesenchymal stem cells promote acute full-thickness skin wound healing and regeneration in mice

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【**Abstract**】 Various types of acute or chronic skin wounds cause physiological and psychological challenges for the patients. After retrospective analysis of Medicare beneficiaries in 2018, about 8.2 million people suffered acute and chronic skin wounds. It is urgent to develop a novel and practical therapy which can effectively prevent the wound infections and accelerate the wound healing in clinical practice. Our previous study demonstrated that thermosensitive hydrogel PF-127 combined with sodium ascorbyl phosphate (SAP) loaded Wharton's jelly mesenchymal stem cells (WJMSCs) can promote the repair and regeneration of both acute full-thickness skin wound and diabetic wound. However, the cell viability and survival rate were still low in the PF-127/SAP combination, which may limit the effectiveness of MSC transplantation in treating skin wounds. To resemble the physiological microenvironment of the native niche in vivo, three-dimensional (3D) encapsulation technique of MSCs has been applied in tissue engineering for a long time, as an important implantation method of stem cells to repair the damaged tissue. 3D culture strategy conditions have been demonstrated to enhance cell-cell interactions and preserve stemness characteristics. MSCs therapy combining with 3D biomaterials encapsulation is currently being investigated as a potential therapeutic avenue for different skin wounds.

In this study, we firstly seeded the WJMSCs into 3D gelatin microspheres (GM) and then encapsulated them with hydrogel PF-127/SAP combination. CCK-8 and EdU assay results showed that WJMSCs seeding in GM increased the cell viability and proliferation ability in vitro. Besides, there were almost no cell death occurred in WJMSCs carried by GM. Loading with GM also improved cell viability and survival rate of WJMSCs which encapsulated with PF-127 and relieved the cytotoxicity of PF-127. In acute full-thickness skin wound, transplantation of WJMSCs/GM/PF-127/SAP promoted the dermis regeneration

via increasing the dermis thickness, newborn hair follicles while decreasing the scar width comparing with other control groups (PBS; GM; WJMSCs; WJMSCs + GM; WJMSCs+PF-127+SAP). WJMSCs/GM/PF-127/SAP transplantation also accelerated the deposition of collagen and increased the ratio of Type III/I collagen fibers at wound site which was helpful to wound scar-free healing. At last, we investigated the potential mechanism of WJMSCs/GM/PF-127/SAP transplantation in promoting acute skin wound healing. The immunohistochemical staining results showed that transplantation of WJMSCs/GM/PF-127/SAP improved the macrophage transformation from proinflammatory M1 type to anti-inflammatory M2 type, cell proliferation, and neovascularization at the wound site to promote the skin wound healing and regeneration.

Collectively, our study reveals a novel and effective system to delivery WJMSCs to promote the acute full-thickness skin wound healing and to prevent the formation of fibrotic scarring. Transplantation of WJMSCs/GM/PF-127/SAP facilitates WJMSCs-mediated acute full-thickness skin wound in mice through promoting the macrophage transformation, cell proliferation, neovascularization and collagen deposition. This strategy not only increased the quality of transplanted WJMSCs but also prolonged the retention time of WJMSCs at wound site, which further improved the therapy effect of WJMSCs transplantation on skin wound. Our findings may potentially provide a helpful therapeutic strategy for patients with skin wound.

【 **Keywords** 】 Gelatin Microspheres; Wharton's jelly mesenchymal stem cells; Hydrogel PF-127; Sodium ascorbyl phosphate; Skin wound

人胚胎间充质干细胞（MSC）减缓小鼠矽肺病理进程的潜在机制研究

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【摘要】目的 探讨人胚胎间充质干细胞（Mesenchymal stem cell，MSC）对减缓小鼠矽肺病理进程的作用及机制。方法 C57BL/6 小鼠 72 只随机分为对照组（NC），模型组（SiO_2），治疗组（SiO_2+MSC），模型组和治疗组分别在第 0d，第 7d 气管滴注 50 mL 50 mg/mL SiO_2 建立小鼠矽肺模型，对照组气管滴注等量生理盐水。建模 14d 后，治疗组经尾静脉注射 0.1 mL 3 ´ 10^6 个 MSC，模型组和对照组经尾静脉注射等量的 PBS。 分别在建模后的 28d、56d 每组各处死 12 只小鼠，收取肺泡灌洗液、血浆和肺组织。ELISA 法分别检测小鼠血浆炎性因子（TNF-α和 IL-6）的表达和肺泡灌洗液中羟脯氨酸含量；HE 和 Masson 染色分别观察肺组织病理变化和胶原沉积；Western blot 和 IF 染色检测上皮-间质转化标志物（α-SMA、N-Ca、Vimentin 和 Collage I），上皮细胞标记物 (Krt5、Krt14、CCSP)以及 Bmi1 信号相关蛋白表达。结果 与对照组相比，治疗组和模型组均出有矽结节，而与模型组小鼠相比，MSC 治疗组中小鼠矽肺中的矽结节数量与大小（H&E）、ECM 沉积（Masson）、炎性因子 TNF-α, IL-6 以及小鼠肺组织的羟脯氨酸含量显著性降低（$P < 0.05$）。同时分子机制进一步探究发现，MSC 可以显著性抑制 EMT 标记物（α-SMA、N-Ca、Vimentin 和 Collage I）降低，并伴随着肺脏上皮细胞标记物，如基底细胞标记物（Krt14，Krt5），分泌细胞标记物 CCSP 以及调控干细胞增值衰老关键蛋白 Bmi1 的表达显著升高。结论 人胚胎间充质干细胞能够减缓小鼠矽肺病理进程，其机制可能通过激活 Bmi1 信号进而激活内源性肺脏干细胞参与肺脏的损伤与修复。

【关键词】 矽肺；间充质干细胞（MSC）；Bmi1，肺脏干细胞

A LncRNA-Encoded Human-specific De Novo Protein Promotes Cortical Expansion and Folding

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【**Abstract**】New genes play key roles in acquisition of evolutionary characteristics such as the neocortex expansion and sulci& gyri formation. Genes are variably generated, among which de novo origination from ancestral non-coding DNAs potentially underwrites the evolutionary novel features of human. Here we report a human-specific de novo protein that is encoded by a long non-coding RNA, is specifically expressed in human fetal brain, preferentially in the ventricular zone. This de novo protein interacts with membrane protein Na/K ATPase (Atp1a1) to regulate brain cell proliferation. In hESC-derived cortical organoids, knock out of de novo protein exhibited compromised human specific developmental features. Knock-in of this long non-coding RNA into mouse instigates an enlarged brain with expanded cortex and sulci& gyri-like structures. Single cell transcriptomes indicate that some cortical progenitors and neurons are generated and amplificated in ways that are unique in human. Additionally, cognitive ability and working memory are improved in transgenic adult mouse. Hence, we identify a human-specific de novo protein that promotes cortical expansion and folding, which facilitates to elucidate the enigmatic function of human brain.

【**Keywords**】 Human-specific, Cortical development, Stem cell, Organoid

Human endometrium-derived stem cell improves cardiac function after myocardial ischemic injury by enhancing angiogenesis and myocardial metabolism

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【**Abstract**】Background: The human endometrium in premenopausal women is an active site of physiological angiogenesis,with regenerative cells present, suggesting that the endometrium contains adult angiogenic stem cells. In the context of cardiac repair after ischemic injury, angiogenesis is a crucial process to rescue cardiomyocytes. We therefore investigated whether human endometrium-derived stem cells (hEMSCs) can be used for cardiac repair after ischemic injury and their possible underlying mechanisms.

Methods: Comparisons were made between hEMSCs successfully isolated from 22 premenopausal women and human bone marrow mesenchymal stem cells (hBMSCs) derived from 25 age-matched patients. Cell proliferation, migration, differentiation, and angiogenesis were evaluated through in vitro experiments,while the ability of hEMSCs to restore cardiac function was examined by in vivo cell transplantation into the infarcted nude rat hearts.

Results: In vitro data showed that hEMSCs had greater proliferative and migratory capacities, whereas hBMSCs had better adipogenic differentiation ability. Human umbilical cord vein endothelial cells, treated with conditioned medium from hEMSCs, had significantly higher tube formation than that from hBMSCs or control medium, indicating greater angiogenic potentials for hEMSCs. In vivo, hEMSC transplantation preserved cardiac function, decreased infarct size, and improved tissue repair post-injury. Cardiac metabolism, assessed by 18F-FDG uptake, showed that 18F-FDG uptake at the infarction area was significantly higher in both hBMSC and hEMSC groups, compared to the PBS control group, with hEMSCs having the highest uptake, suggesting hEMSC treatment improves cardiomyocyte metabolism and survival after injury. Mechanistic assessment of the angiogenic potential for hEMSCS revealed that angiogenesis-related factors angiopoietin 2, Fms-like tyrosine kinase 1, and FGF9 were significantly upregulated in hEMSC-implanted infarcted hearts, compared to the PBS control group.

Conclusion: hEMSCs, compared to hBMSCs, have greater capacity to induce angiogenesis, and improved cardiac function after ischemic injury.

【 **Keywords** 】 Human endometrium-derived stem cells, Myocardial ischemic injury, Human bone marrow mesenchymal stem cells, Angiogenesis, Cardiac repair

缺血性脑卒中急性期 SerpinA3N 调控小胶质细胞稳态研究

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【摘要】小胶质细胞与星型胶质细胞是脑内炎症反应的重要调控细胞，二者的相互作用在炎症反应急性期具有高度动态、过程复杂、级联放大的特点，是进行脑损伤干预的重点方向。小胶质细胞可以通过 IL1-α、TNF 和 C1q 三种炎症因子激活星型胶质细胞杀伤神经元。但是炎症状态下，小胶质细胞如何被动态和精准调控尚不够明确。我们前期发现，一个具有潜在抑制小胶质细胞激活功能的丝氨酸蛋白酶抑制因子 SerpinA3N，在脑卒中急性期的星型胶质细胞中表达显著升高，提示星型胶质细胞来源的 SerpinA3N 可能参与炎症反应中调控小胶质细胞激活。我们利用小鼠脑卒中模型，结合脑立体定位注射、基因编辑等技术，研究星型胶质细胞来源的 SerpinA3N 在脑卒中不同时期的表达特点，对小胶质细胞激活的调控，以及在不同时期、不同疾病状态下 SerpinA3N 如何时期和状态特异性的调控脑卒中的发生、发展和转归，从而开发急性脑卒中干预新策略。

【关键词】 缺血性脑卒中 小胶质细胞 星型胶质细胞

Functional otic neuronal organoids derived from human embryonic stem cells

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【**Abstract**】Spiral ganglion neurons (SGNs) dysfunction of mammalian cochlea takes the leading cause of sensorineural hearing loss. SGNs are sensitive to ototoxic insults such as drugs, noises and aging, and usually needs cooperative development of other cell lineages. To facilitate the long-term survival and normal function of SGNs are of great importance. Here, we constructed human otic neuronal organoids (hONOs) in a *de novo* 3D and self-organized manner after the developmental principle of otic neurons. hESCs were step-wisely induced toward otic-placode ectoderm, otic neuronal progenitor and mature phases, respectively. Gene expression profiles of these oganoids accordingly exhibited a temporally transition from the early, intermediate and the late ones. Mature hONOs (differentiation day 35 to 160, D35-160) were composed of SGN-like neuronal cells and neurotrophic glial cells. SGNs in mature hONOs expressed otic neuronal markers such as MAP2, NEUROG1, NEUROG2, CHGB, CALB1, CALB2, NEFL, vGLUT2, vGLUT3, TrkB, and TrkC. Glial markers such as SOX2, SOX9, POU3F4 and VIM, and genes encoding extracellular matrix molecules such as COL3A1, COL5A2 and ITGA6, were also highly expressed in these organoids. Spontaneous giant depolarized potential (GDP)-like events were detected in early and intermediate hONOs. Glutamic acid potentiates the GDP-like events, and glutamatergic network inhibition strongly decreased the corresponding indexes in hONOs at mature stages. We thus established functional hONOs bearing cellular heterogeneity and neuronal activity, which provides unique tools to study the development and function of human auditory system. An in-depth analysis of the temporal maturation of hONOs also might facilitate the decoding of signals from cochlear implants.

【**Keywords**】inner ear; human embryonic stem cells; 3D differentiation; otic neuronal organoids; action potentials

Human umbilical cord derived mesenchymal stem cells transfer oncolytic reovirus to tumor cells via extracellular vesicle

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【**Abstract**】Oncolytic viruses are powerful novel agents for suppressing tumor growth. Similar to other oncolytic viruses, reovirus is exposed to antiviral immunity in vivo when administered intravenously, which greatly reduces the anti-tumor efficacy. To overcome this obstacle, human umbilical cord mesenchymal stem cells (UC-MSCs) were used as cellular vehicles to deliver oncolytic reovirus. Transmission electron microscopy and indirect immunofluorescence staining were used to verify the presence of reovirus in the cytoplasm of UC-MSCs. Transwell co-culture experiments showed that reovirus released by UC-MSCs was able to pass through a 0.4 μm porous membrane and infect tumor cells in the presence of neutralizing antibodies. The virus was detected in extracellular vesicles (EVs) derived from pre-infected UC-MSCs, and virus-encapsulating EVs had a killing effect on tumor cells. Collectively, the results indicate that UC-MSCs transfer reovirus to tumor cells via EV release, thus, providing a theoretical basis for the use of UC-MSCs as carriers of oncolytic reovirus.

【**Keywords**】Human umbilical cord-derived mesenchymal stem cells, oncolytic reovirus, transfer, extracellular vesicle, neutralizing antibodies

Mechanism of Proinflammatory Factor NLRC3 Signaling Regulates Embryonic Hematopoietic Stem Cell Production and Development

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【 **Abstract** 】 Hematopoietic stem cells (HSCs) of vertebrates are emerged from hematopoietic endothelium (HE) cells located in the aorta-gonard mesonephros (AGM) through endothelial to hematopoietic transition (EHT) during the embryonic period. The process is not only affected by the microenvironment, but also regulated by a variety of transcription factors, cytokines, and epigenetic regulatory factors that work together as a network through various signaling pathways. In recent years, TNFa, IFNr, TLR4, and IL6 have all been reported to regulate vertebrate embryonic HSCs.

In this study, we first used sequencing data from multiple public databases to compare the expression differences between hematopoietic endothelial cells and hematopoietic stem cells. Secondly, by analyzing corresponding differentiation stage in the system of inducing mouse embryonic stem cell (mESC) and hESCs in vitro, we anchored NLRC3 as the research object based on the size of the difference and gene conservation. NLRC3 is a pattern recognition receptors (PRRs), belonging to the family of nucleotide binding oligomerization domain like receptors (NLRs), but the signal regulates embryonic HSCs production, proliferation, differentiation and development and its possible mechanism is still lack of clear data.

Consequently, we used the microinjection Morpholino (MO) and CRISPR-Cas9 technology to knock down and out NLRC3 in zebrafish embryos and observed different parts of embryos at different developmental time points (hours postfertilization, hpf). Compared with the control group, with confocal array, in situ hybridization, real-time fluorescent quantitative PCR (Quantitative Real-time PCR) and other technical methods to confirm that NLRC3 zebrafish mutants also have HSCs deletion and downstream differentiation decreased.

Further experiments found that NLRC3 knockout did not affect the primitive wave of hematopoiesis in zebrafish, nor did it affect the vascular development of zebrafish, nor was it accompanied by significant changes in apoptosis signals. In order to explore the underlying

mechanism leading to the loss of hematopoiesis, we use fluorescence-activated cell sorting technology to isolate HEs from transgenic zebrafish that use fluorescence to label hematopoietic endothelial cells and hematopoietic stem cells for deep sequencing.

According to the analysis of sequencing data, we used microinjection, in situ hybridization, qPCR and other methods to verify that NLRC3 is located upstream of Notch1a, and the results of HSCs being regulated by Notch downstream target gene Hey1. According to previous literature reports, we found that NLRC3 is located downstream of NF-kB by means of targeted inhibition and mRNA overexpression in vitro, confirming that NLRC3 is a part of the regulatory network from NF-kB to Notch, and is involved in the regulation of zebrafish embryonic hematopoiesis.

In order to further verify our findings in higher model animals, we used CRISPR-Cas9 technology and non-homologous recombination to repair the introduced mutations to construct NLRC3 knockout mice. Combined with the phenotype of the model animal zebrafish, this project will focus on the hematopoietic stage of the fetal liver in the embryonic mice. It was found that the number of HSCs in the fetal liver of knockout mice decreased and the number of blood cells in downstream lines decreased. To verify the in vivo function of embryonic hematopoietic stem cells, we performed bone marrow transplantation experiments. The chimerism rate and the differences in blood cells of each line further confirmed that hematopoietic abnormalities in NLRC3 knockout mice were mainly due to the internal damage of hematopoietic stem cells, rather than the influence of the extracellular environment.

In summary, this project uses cells, zebrafish and mice, to deeply explore the relationship between inflammatory signaling pathways and embryonic hematopoiesis. Studies have found that NLRC3 regulates the generation and differentiation of HSCs in early embryos, and its regulatory pathway is downstream of NF-kB, and it plays a role through Notch and its downstream target gene Hey1.

【Keywords】 embryonic hematopoietic stem cells, hematopoietic stem cells, zebrafish, fetal liver, proinflammatory factors, pattern recognition receptor, NLRC3

负载干细胞源性外泌体的复合支架促进糖尿病骨再生及机制研究

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【摘要】糖尿病可引起骨髓间充质干细胞（BMSCs）功能改变，从而导致成骨能力下降、骨代谢失衡，严重影响骨再生。无细胞组织工程研究表明，干细胞源性外泌体具有较强的促进骨组织再生潜力，但直接使用易流失、效率低，且促进糖尿病环境下骨组织再生的作用和机制尚不清楚。本课题通过 3D 打印技术构建出明胶/透明质酸/纳米羟基磷灰石复合支架，该支架孔径控制在 415.9 ± 21.35 μm，孔隙率 77.82%，4 周降解率可达 40%，同时能促进细胞黏附及增殖，具有良好的生物相容性。通过表面涂覆聚赖氨酸的静电相互作用以及支架固有的空间壁垒作用，实现外泌体的缓释，延长其体内作用时间。利用高糖 DMEM 培养基（葡萄糖含量 4.5 g/L）模拟高糖环境，体外细胞实验表明，高糖可抑制 BMSCs 的成骨分化能力，而外泌体的加入可逆转高糖对 BMSCs 成骨分化能力的损伤。通过建立糖尿病大鼠颅骨临界缺损模型，深入研究该复合支架对糖尿病大鼠骨缺损的修复效果，并探讨外泌体调控 BMSCs 功能促进糖尿病骨再生的作用机制。本课题有望利用外泌体逆转糖尿病引起的细胞功能改变，促进糖尿病患者骨再生，为无细胞组织工程骨修复提供新的治疗策略。

【关键词】 骨髓间充质干细胞；外泌体；糖尿病；骨再生

一条参与结合 NR2E1 的 LSD1 多肽对脑癌干细胞的抑制

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【摘要】脑癌干细胞因为其对化疗和放疗具有抗性，与脑癌复发快和预后极差密切相关。因此，我们研究靶向脑癌干细胞，通过研究脑癌干细胞的增殖机制，有针对性地设计多肽干扰相关机制，以实现抑制脑癌干细胞生长的目的，从而为脑癌的治疗提供新的策略。

采用从 Nestin-TV-a mice 小鼠分离出来的脑癌干细胞，我们发现在小鼠脑癌干细胞中孤儿核受体 NR2E1 招募赖氨酸特异性组蛋白去甲基化酶 LSD1 到抑癌基因 Pten 的启动子区域，通过对其组蛋白 H3K4me 和 H3K4me2 去甲基化而抑制 Pten 的表达，从此促进脑癌干细胞的增殖。通过 Hydrogen/Deuterium Exchange and Mass Spectrometry (HDX-MS)实验，发现一条位于 LSD1 蛋白 SWIRM 结构域多肽主要参与 NR2E1 和 LSD1 的结合，并形成 LSD1 蛋白 SWIRM 结构域特异的蛋白结合口袋。在小鼠脑癌干细胞中过表达这条多肽能够干扰 NR2E1 和 LSD1 所促进的脑癌干细胞增殖，但不会影响 NR2E1 和 LSD1 都高表达的 293T 细胞的增殖。进一步在人脑癌干细胞中诱导该多肽的表达，我们发现该多肽能够有效抑制人脑癌干细胞在体外增殖和体内成瘤。

我们的研究揭示 NR2E1 和 LSD1 协同促进脑癌干细胞增殖。针对 NR2E1 和 LSD1 的结合，我们发现了一条有效抑制脑癌干细胞增殖的多肽。这条多肽有可能成为脑癌多肽药物开发的先导肽。

【关键词】 脑癌干细胞，NR2E1,LSD1,多肽

HIRA 复合体在胚胎干细胞中抑制反转座子表达

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【摘要】HIRA 复合体是介导 H3.3 组蛋白变体沉积到染色体上的关键组蛋白伴侣。但是，它是否参与转座子的表达调控仍然未知。我们通过 shRNA 筛选了 HIRA 复合体成员在胚胎干细胞中抑制转座子表达的功能。我们发现 Hira、Ubn2 与 Ubn1 是转座子的重要抑制蛋白。我们意外地发现 Ubn2 和 Hira 可以靶向不同的反转座子家族，虽然它们也共同靶向一些基因。我们进一步发现 Ubn2 而非 Hira 可以抑制胚胎干细胞获得 2 细胞样细胞的状态或者分化成滋养外胚层干细胞。在机制上，Ubn2 和 Hira 通过调控 H3.3 在染色体上的沉积来抑制反转座子表达。Ubn2 或 Hira 减少导致的 H3.3 沉积下降最终引起反转座子抑制标记 H3K9me2 与 H3K9me3 的减少。总而言之，我们的发现揭示了 HIRA 复合体不同成员在胚胎干细胞中调控反转座子表达时的独特功能。

【关键词】 胚胎干细胞；反转座子；HIRA

PI3K inhibitor impairs tumor progression and enhances sensitivity to anlotinib in anlotinib-resistant osteosarcoma

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【**Abstract**】Despite recent improvements in the therapeutic management of osteosarcoma (OS), ongoing challenges in overcoming the resistance to tyrosine kinase inhibitors (TKIs) warrants new strategies still needed to improve overall patient survival. In this study, we established 4 anlotinib-resistant OS cell lines and demonstrated the mechanism of anlotinib resistance is due to the loss of PTEN and the reactivation of the MAPK pathway. Reduced PTEN expression was also seen in tumor samples from OS patient with lung metastasis. We investigated the effects of an orally available PI3K inhibitor on the progression of the resistant cells and xenograft nude mouse model, either alone or in combination with anlotinib. Notably, PI3K inhibitor suppressed anlotinib resistant OS cell proliferation, migration, invasion, and cytoskeleton formation, and induced apoptosis. Combination with anlotinib augmented these effects that invited by restoring PTEN expression and decreasing MAKP and PI3K/AKT/mTOR signaling. We found that PI3K inhibitor can reverse anlotinib resistance in OS, limiting OS cell development in combination with anlotinib. Our findings rationalize further study of the applications of PI3K inhibitor that can be clinically used in anlotinib refractory OS management.

【**Keywords**】anlotinib, osteosarcoma, resistance, PTEN, tyrosine kinase inhibitors

miR-135a Reduces Osteosarcoma Pulmonary Metastasis by Targeting Both BMI1 and KLF4

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【**Abstract**】Because of the modest response rate after surgery and chemotherapy, treatment of osteosarcoma (OS) remains challenging due to tumor recurrence and metastasis. miR-135a has been reported to act as an anticarcinogenic regulator of several cancers. However, its expression and function in osteosarcoma remain largely unknown. Here, we reported that abridged miR-135a expression in OS cells and tissues, and its expression is inversely correlated with the expression of BMI1 and KLF4, which are described as oncogenes in several cancers. Ectopic expression of miR-135a inhibited cell invasion and expression of BMI1 and KLF4 in OS cells. In vivo investigation confirmed that miR-135a acts as a tumor suppressor in OS to inhibit tumor growth and lung metastasis in xenograft nude mice. BMI1 and KLF4 were revealed to be direct targets of miR-135a, and miR-135a had a similar effect as the combination of si-BMI1 and si-KLF4 on inhibiting tumor progression and the expression of BMI1 and KLF4 in vivo. Altogether, our results demonstrate that the targeting of BMI1/KLF4 with miR-135a may provide an applicable strategy for exploring novel therapeutic approaches for OS.

【**Keywords**】 osteosarcoma, metastasis, KLF4, BMI1, microRNA

Chloroquine suppresses proliferation and invasion and induces apoptosis of osteosarcoma cells by inhibiting the phosphorylation of STAT3

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【**Abstract**】Objective: Osteosarcoma (OS) is characterized by a high rate of metastasis. It has been found that tumor cells can bypass apoptosis which leads to an uncontrolled proliferation, but chloroquine (CQ) can have effect on the tumors by inducing apoptosis. We aimed to explore the effects and the hypothetical mechanism of CQ effects on OS.

Methods: We first estimated the CQ effects on proliferation, apoptosis, migration, invasion, and lamellipodia formation of OS cells. Mice bearing xenograft model were used to test the anti-tumor growth and lung metastasis effects of CQ in OS. Western blot and immunohistochemistry were used to explore the mechanism of CQ effects and the association between p-STAT3 expression and lung metastasis of OS patients.

Results: CQ induces the apoptosis and suppressed the viability, proliferation, migration, invasion, and lamellipodia formation of OS cells in vitro. In vivo experiments demonstrated that CQ inhibited tumor growth and lung metastasis. The lung metastasis was associated with the p-STAT3 expression in OS patients.

Conclusion: CQ inhibited progression of OS cells in vitro, and suppressed tumor growth and lung metastasis in vivo. p-STAT3 can be a predictive biomarker for lung metastasis in osteosarcoma patients.

【**Keywords**】 chloroquine, osteosarcoma, apoptosis, metastasis, p-STAT3

Continuous expression of reprogramming factors induces and maintains mouse pluripotency without specific growth factors and signaling inhibitors

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【 **Abstract** 】 Objectives Derivation and maintenance of pluripotent stem cells (PSCs) generally require optimized and complex culture media, which hinders the derivation of PSCs from various species. Expression of Oct4, Sox2, Klf4, and c-Myc (OSKM) can reprogram somatic cells into induced PSCs (iPSCs), even for species possessing no optimal culture condition. Herein, we explored whether expression of OSKM could induce and maintain pluripotency without PSC-specific growth factors and signaling inhibitors.

Methods The culture medium of Tet-On-OSKM/Oct4-GFP mouse embryonic stem cells (ESCs) was switched from N2B27 with MEK inhibitor, GSK3β inhibitor, and leukemia inhibitory factor (LIF) (2iL) to N2B27 with doxycycline. Tet-On-OSKM mouse embryonic fibroblast (MEF) cells were reprogrammed in N2B27 with doxycycline. Cell proliferation was traced. Pluripotency was assessed by expression of ESC marker genes, teratoma, and chimera formation. RNA-Seq was conducted to analyze gene expression.

Results Via continuous expression of OSKM, mouse ESCs (OSKM-ESCs) and the resulting iPSCs (OSKM-iPSCs) reprogrammed from MEF cells propagated stably, expressed pluripotency marker genes, and formed three germ layers in teratomas. Transcriptional landscapes of OSKM-iPSCs resembled those of ESCs cultured in 2iL and were more similar to those of ESCs cultured in serum/LIF. Furthermore, OSKM-iPSCs contributed to germline transmission.

Conclusions Expression of OSKM could induce and maintain mouse pluripotency without specific culturing factors. Importantly, OSKM-iPSCs could produce gene-modified animals through germline transmission, with potential applications in other species.

【 **Keywords** 】 Reprogramming factors, Culture medium, Self-renewal, Pluripotency, Germline transmission, Gene-modified animals

ELTD1 deletion enhances the hematopoietic differentiation of human embryonic stem cells

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【**Abstract**】Background and purpose Recent years have seen an explosion in the therapeutic advance of chemotherapy and radiotherapy in hematologic disorders, however, they provide limited relief with an undefined prognosis. With few side effects, hematopoietic stem cells (HSCs) transplantation is the best solution for the treatment of several hematologic malignancies and autoimmune diseases currently. Due to the limited source of HSCs, the number of HSCs for clinical use is far from enough. Given the multiple differentiation potential of human embryonic stem cells (hESCs), it is an optimal way to generate sufficient HSCs from hESCs to meet clinical needs. Hence, there is an urgent need to study the underlying mechanism of hematopoietic differentiation of hESCs.

Methods In this study, ELTD1 homozygous knockout human embryonic stem cell line was generated by the iCRISPR/Cas9 system to make better understanding of ELTD1 in hematopoiesis. Western Blot, qPCR and immunofluorescence staining were used to detect the expression of pluripotent makers. Teratoma formation was performed to assess the differentiation ability of three germ layers in vivo. Flow cytometry, qPCR, were applied to detect the expression of specific markers, including CD309, APLNR, CD31, CD34, CD43 and CD45 during the hematopoietic differentiation. Immunofluorescence was performed to investigate the expression of CD309, CD31, CD34 and CD43.

Results We found that ELTD1 (Epidermal growth factor, latrophilin and seven-transmembrane domain-containing 1), an orphan G-protein-coupled receptor (GPCR) plays a key role during the hematopoietic differentiation of hESCs. The generated ELTD1 homozygous knockout human embryonic stem cell line was confirmed with normal karyotype, typical undifferentiated morphology, pluripotency and trilineage differentiation potential in vitro. The flow cytometry results showed that ELTD1-/- hESCs generated more CD31+, CD34+ cells at day 6, CD43+ cells at day 9 and CD45+ cells at day 12 compared with WT cells. The similar results were also confirmed by qPCR. Consistently, more expression of CD31, CD34 at day 6 and CD43 at day 9 were verfied by immunofluorescence staining.

Conclusion It was found that ELTD1 knockout could significantly promote the generation of hemogenic endothelium so as to facilitate the subsequent hematopoietic differentiation of hESCs in vitro.

【**Keywords**】ELTD1, Human embryonic stem cells, Hematopoietic differentiation

RGD 三维体系促进稳态外周血中稀有循环造血干祖细胞再生

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【**Abstract**】 A spectrum of hematopoietic disorders has been successfully treated by transplantation of hematopoietic stem/progenitor cells (HSPCs), which are either isolated from umbilical cord blood (UCB) or mobilized from bone marrow into the peripheral blood by mobilization reagents such as G-CSF and CXCR4 antagonist. However, clinical application of HSC transplantation is still impeded by low amount of UCB HSCs and high risk of mobilization failure. Whether circulating HSPCs (cHSPCs) in peripheral blood (PB) could be used as an alternative source and expanded to collect enough HSCs for transplantation remain largely elusive. Here, we developed a three-dimensional culture system (3DCS) consisting of self-assembled amino acid polypeptide of arginine, glycine, and aspartate (RGD) and a series of cytokines and small molecules (SCF, FLT3L, TPO, IL-3, IL-6, VEGF, SR1 and Vc), and found that the frequency and cell number of $CD34^+$ cells increased by 125- and 70-fold respectively after 14 days culture in 3DCS compared to day 0, suggesting that 14 days culture of appropriately 212 ml of PB could obtain 1.5×10^8 $CD34^+$ cells and provide enough HSPCs for transplantation into an adult with 75 kg body weight. Further, we demonstrated that the expanded cHSPCs in 3DCS exhibited both long-term self-renewal and multilineage differentiation capacities through limiting dilution competitive repopulating unit assays and serial transplantation assays. Mechanistically, high-throughput qRT-PCR revealed that 3DCS-derived cHSPCs showed similar transcriptome profiles to those of bone marrow (BM)-derived $CD34^+$ HSPCs. Single-cell RNA-Seq elucidated that 3DCS fabricated the HSC microenvironment including HSPCs, committed progenitors and mature lineage cells such as macrophages and Treg cells, which secreted cytokines such as TNF-α to support cHSPC survival. Finally, we validated that 3DCS could also promote the expansion of cHSPCs in patients who previously failed in HSC mobilization. In conclusion, our new 3D culture system mimicks an artificial niche to successfully expand rare cHSPCs in peripheral blood and maintain their reconstitution abilities, which provides an alternative source for the clinical application of HSC therapy, particularly for the patients/donors who have failed to show HSC mobilization.

【**Keywords**】 Hematopoietic stem/progenitor cell transplantation; peripheral blood mononuclear cells; expansion; mobilization; three-dimensional culture; self-renewal and multilineage differentiation.

Enhancing targeted transgene knock-in by donor recruitment

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【**Abstract**】With the development of the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-based gene editing technology, obtaining gene-knockout cells or animal models is becoming increasingly convenient. However, other genomic DNA manipulations, including targeted transgene integration or replacement, remain challenging, especially for treatment of disease with gene therapy. Homology-mediated end joining (HMEJ) and homology-independent targeted integration (HITI) have been reported to increases the efficiency of targeted gene integration, but the results are far from treatment efficiently in vivo. Here, we present a strategy for enhanced HMEJ (enHMEJ) and enhanced HITI (enHITI) by fusing Cas9 with a specific DNA-binding protein, integrase p32, from the human immunodeficiency virus type 1 (HIV-1), to recruit donors harboring long terminal repeats (LTRs) to the DSB site to increase the targeted integration efficiency by 3-fold. Via continuous expression of OSKM, mouse ESCs (OSKM-ESCs) and the resulting iPSCs (OSKM-iPSCs) reprogrammed from MEF cells propagated stably, expressed pluripotency marker genes, and formed three germ layers in teratomas. Transcriptional landscapes of OSKM-iPSCs resembled those of ESCs cultured in 2iL and were more similar to those of ESCs cultured in serum/LIF. Furthermore, OSKM-iPSCs contributed to germline transmission.

【**Keywords**】 Enhancing, Gene targeting, Integrase, Safety

人子宫内膜来源的干细胞通过增强血管生成和心肌代谢来改善心肌缺血损伤后的心功能

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Title：Human endometrium-derived stem cell improves cardiac function after myocardial ischemic injury by enhancing angiogenesis and myocardial metabolism

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【**Abstract**】Contents:Background: The human endometrium in premenopausal women is an active site of physiological angiogenesis,with regenerative cells present, suggesting that the endometrium contains adult angiogenic stem cells. In the context of cardiac repair after ischemic injury, angiogenesis is a crucial process to rescue cardiomyocytes. We therefore investigated whether human endometrium-derived stem cells (hEMSCs) can be used for cardiac repair after ischemic injury and their possible underlying mechanisms.

Methods: Comparisons were made between hEMSCs successfully isolated from 22 premenopausal women and human bone marrow mesenchymal stem cells (hBMSCs) derived from 25 age-matched patients. Cell proliferation, migration, differentiation, and angiogenesis were evaluated through in vitro experiments,while the ability of hEMSCs to restore cardiac function was examined by in vivo cell transplantation into the infarcted nude rat hearts.

Results: In vitro data showed that hEMSCs had greater proliferative and migratory capacities, whereas hBMSCs had better adipogenic differentiation ability. Human umbilical cord vein endothelial cells, treated with conditioned medium from hEMSCs, had significantly higher tube formation than that from hBMSCs or control medium, indicating greater angiogenic potentials for hEMSCs. In vivo, hEMSC transplantation preserved cardiac function, decreased infarct size, and improved tissue repair post-injury. Cardiac metabolism, assessed by 18F-FDG uptake, showed that 18F-FDG uptake at the infarction area was significantly higher in both hBMSC and hEMSC groups, compared to the PBS control group, with hEMSCs having the highest uptake, suggesting hEMSC treatment improves cardiomyocyte metabolism and survival after injury. Mechanistic assessment of the angiogenic potential for hEMSCS revealed that angiogenesis-related factors angiopoietin 2, Fms-like tyrosine kinase 1, and FGF9 were significantly upregulated in hEMSC-implanted infarcted hearts, compared to the PBS control group.

Conclusion: hEMSCs, compared to hBMSCs, have greater capacity to induce angiogenesis, and improved cardiac function after ischemic injury.

【**Keywords**】Human endometrium-derived stem cells, Myocardial ischemic injury, Human bone marrow mesenchymal stem cells, Angiogenesis, Cardiac repair

人子宫内膜来源的干细胞，心肌缺血性损伤，人骨髓间充质干细胞，血管生成，心脏修复

利用可诱导自杀策略建立多能干细胞治疗的安全调控系统

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【摘要】多能干细胞 (PSC) 在再生医学领域中具有广阔的应用前景。而 PSC 治疗在临床中的一个主要问题是在治疗的过程中由于未分化干细胞的污染而形成畸胎瘤。组成型启动子或内源性 Sox2 启动子已被用于驱动诱导型 caspase-9 (iCasp9) 基因表达，但不能专门根除未分化的 PSC。在这里，我们将 iCasp9 基因插入到人和小鼠 PSC 的内源性 OCT4 基因座中，而不影响它们的多能性。二聚化 (CID) 的化学诱导剂 AP1903 可诱导 Casp9 活化，从而导致体外和体内特定未分化 PSC 的凋亡。由于内源性 OCT4 基因的沉默，分化的细胞谱系得以存活。当在免疫缺陷小鼠中注射 PSC，并在 2 周内施用 CID 时，人和小鼠的 PSC 生长是可控的。然而，超过 2 周的给药会导致畸胎瘤形成和小鼠死亡，因为大量体细胞已经从 PSC 分化出来。总之，我们开发了一种特异且高效的 PSC 自杀系统，能够更好的克服再生医学背景下的 PSC 的肿瘤发生。

【关键词】多能干细胞（PSCs）；诱导型 caspase-9 (iCasp9)；OCT4；畸胎瘤

Generation of integration-free induced pluripotent stem cell models for Turner syndrome patients with cognitive deficit and left ventricular hypertrophy

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【**Abstract**】(Background) Less than 1% of 45,X monosomy (Turner syndrome, TS) fetuses can survive through the pregnancy. However, the surviving TS patients still suffer from increased morbidity and mortality, with diverse symptoms from infertility, heart abnormalities, cognitive deficits to other manifestations. How loss of one entire X chromosome drives these conditions remains largely unclear. In this study, we aim to create a panel of induced pluripotent stem cell (iPSC) lines from peripheral blood mononuclear cells (PBMCs) of adult TS patients with cognitive deficit or left ventricular hypertrophy. These patient-specific iPSC lines possess pluripotency and can be differentiated into neurons and cardiomyocytes in vitro, which could be useful tools for modeling nervous system disorders and heart diseases of TS adults. (Methods) After obtaining informed consents, PBMCs were isolated from 2 adult TS patients, one with cognitive deficit (TS1) and the other with left ventricular hypertrophy (TS2). We generated TS1- and TS2-iPSC lines from the PBMCs using non-integrating Sendai viral vectors expressing reprogramming factors Oct3/4, Sox2, c-Myc, and Klf4. The identities of the iPSC lines were confirmed by karyotyping and DNA fingerprint analysis. The pluripotency of the iPSC lines were validated by embryonic body (EB) formation in vitro and teratoma formation in vivo. TS-neurons were derived from the TS-iPSC lines using the Gibco PSC Neural Induction Medium, and the efficacy was evaluated by immunostaining for NeuN and Tau. TS-cardiomyocytes were derived from TS-iPSC lines using the Gibco PSC Cardiomyocyte Differentiation Kit, and the efficacy was evaluated by immunostaining for α-actinin, cTnT and TNNT2. (Results) Both TS1- and TS2-iPSC lines maintain the 45,X karyotype of the donors. Both iPSC lines exhibited strong alkaline phosphatase (AP) activity and expressed human embryonic stem cell (hESC) markers, and could be differentiated into various somatic cell types of the three germ layers both in vitro and in vivo. Furthermore, both iPSC lines were capable of differentiating into neurons and cardiomyocytes. We have developed a protocol not requiring complicated EB formation and able to generate neurons with long axons extended out of the cluster in 20 days, and with positive staining of NeuN and Tau. In addition, we could generate functional cardiomyocytes from TS iPSCs in 14 days, with positive staining of α-actinin, cTnT and TNNT2. (Conclusions) Our study offers unprecedented cellular models to investigate the profound symptoms such as nervous system and cardiovascular diseases of TS adults, and serve as useful tools to develop therapeutic approaches for such diseases.

【**Keywords**】Turner syndrome, induced pluripotent stem cell, chromosomal abnormality, nervous system disorder, heart disease.

Pharmacological regulation of tissue fibrosis by targeting the mechanical contraction of myofibroblasts

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【**Abstract**】 Fibrosis can occur in almost all tissues and organs, and affects normal physiological function, which may have serious consequences, such as organ failure. However, there are currently no effective, broad-spectrum drugs suitable for clinical application. Revealing the process of fibrosis is an important prerequisite for the development of new therapeutic targets and drugs. Studies have shown that the limitation of myofibroblast activation or the promotion of their elimination can ameliorate fibrosis. However, it has not been reported whether a direct decrease in cell contraction can inhibit fibrosis in vivo. Here, we have shown that (-)-blebbistatin (Ble), a non-muscle myosin Ⅱ inhibitor, displayed significant inhibition of liver fibrosis in different chronic injury mouse models in vivo. We found that Ble reduced the stiffness of fibrotic tissues from the early stage, which reduced the extent of myofibroblast activation induced by a stiffer extracellular matrix (ECM). Moreover, Ble also reduced the activation of myofibroblasts induced by TGF-β1, which is the most potent pro-fibrotic cytokine. Mechanistically, Ble reduced mechanical contraction, which inhibited the assembly of stress fibers, decreased the F/G-actin ratio, and led to the exnucleation of YAP1 and MRTF-A. Finally, we verified its broad-spectrum antifibrotic effect in multiple models of organ fibrosis. Our results highlighted the important role of mechanical contraction in myofibroblast activation and maintenance, rather than just a characteristic of activation, suggesting that it may be a potential target to explore broad-spectrum drugs for the treatment of fibrotic diseases.

【**Keywords**】 Fibrosis, Myofibroblasts, Contraction, Inhibitor

Rif1 functions in complex with PRC1.6 to maintain a repressive epigenetic state

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【**Abstract**】The mammalian genome has accumulated a large number of remnants of infectious viruses and other genetic components in the course of evolution, which were called repetitive elements. In mice, nearly half of the genome belongs to repetitive elements. In the process of the conversion of embryonic stem cells to 2C-like cells, in addition to changes in the transcription of some genes, the repetitive elements, which were previously regarded as "junk" elements, actually also undergo certain changes. Here, we intend to investigate the reprogramming process from the two aspects of genetic and repetitive elements changes. First, we analyzed the RNA-seq data of relative genes that can regulate the transition and it's proved that some genes and repetitive elements regulated by Rif1 are similar to that of Pcgf6 and RNF2. Our further analysis of Rif1 and Pcgf1-6 also showed that Rif1 is only highly related to Pcgf6 in terms of regulatory genes (especially the 2-cell marker genes) and repetitive elements. We also find Rif1 can interact with the PRC1.6 complex, and the lack of Rif1 can destroy the integrity of the PRC1.6 complex. Further studies have shown that Rif1 and PRC1.6 complex co-bind to certain chromatin regions and the deletion of Rif1 affects the level of enrichment of PRC1.6 complex at the TSS regions of Rif1 bound genes and repetitive elements. Altogether, our findings suggested that the PRC1.6 complex could repress 2-cell marker genes and repetitive elements through the interaction with Rif1, which could limit the reprogramming of pluripotent ESCs to totipotent 2C-like cells.

【**Keywords**】2C-like cells, repetitive elements, Rif1, PRC1.6 complex

Polycomb-group ring finger 6 controls human pluripotent stem cell lineage commitment by activating SOX2 expression and repressing WNT signaling pathway

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【**Abstract**】Polycomb group (PcG) proteins are known to repress developmental genes during embryonic development and tissue homeostasis. However, emerging evidence suggests that PcG proteins could also function in transcriptional activation. Here, we report that Polycomb group ring finger 6 (PCGF6) controls neural ectoderm specification of human pluripotent stem cells (PSCs) by activating SOX2 gene. Although PSCs with PCGF6 depletion keep the self-renewal capacity intact, they display impaired neural ectoderm differentiation coupled with increased mesendoderm outcomes. Transcriptome analysis revealed that de-repression of the WNT/β-catenin signaling pathway is responsible for the differentiation of PSC toward the mesendodermal lineage. Interestingly, PCGF6 and MYC directly interact and co-occupy a distal regulatory element of SOX2 to activate SOX2 expression, which likely accounts for the defects in neuroectoderm differentiation. Supporting this notion, genomic deletion of the SOX2-regulatory element could phenocopy the impaired neuroectoderm differentiation, while overexpressing SOX2 could rescue the neuroectoderm phenotype caused by PCGF6-depletion. Together, our study reveals that PCGF6 can function as lineage switcher between mesendoderm and neuroectoderm in human PSCs by both suppression and activation mechanism.

【**Keywords**】 PCGF6, human pluripotent stem cells, neural ectoderm differentiation, lineage specification, SOX2, WNT

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