荧光定量PCR仪与基因表达检测

复旦大学洪尚宇教授课题组于*Nature Metabolism*杂志上发表题为“**Ketogenic diet-induced bile acids protect against obesity through reduced calorie absorption**”的文章（Nat Metab. 2024 Jul;6(7):1397-1414）。在研究过程中，洪尚宇教授课题组利用国重平台的荧光定量PCR仪（2014-life-Q7)-384孔板检测了受生酮饮食（KD）调节的循环牛磺脱氧胆酸（TDCA）和牛磺糖去氧胆酸（TUDCA）水平对回肠和Caco-2细胞中碳酸酐酶1（Car1）表达的影响。同时，测定了肠道Farnesoid X receptor信号相关基因和肝脏胆汁酸合成基因的表达情况。本研究揭示了一种独特的宿主-肠道微生物群代谢相互作用机制，即KD降低了编码胆汁盐水解酶（BSH）的肠道细菌*Lactobacillus murinus ASF361*的丰度，从而升高小鼠血清中TDCA和TUDCA的水平并抑制CAR1的表达以降低体重和空腹血糖水平。

QuantStudio™ 7 Flex
实时荧光定量PCR仪

Biorad ddPCR
微滴式数字PCR仪

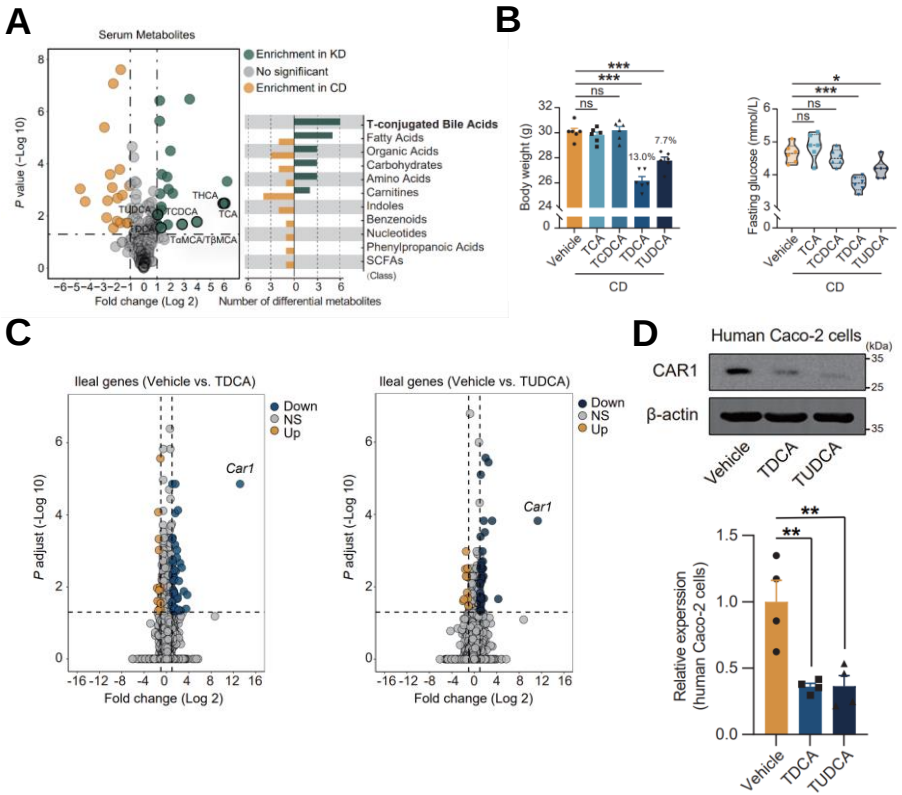

图 (A) 常规饮食和生酮饮食小鼠的差异血清代谢物的火山图。(B) TDCA和TUDCA处理对常规饮食小鼠的体重和空腹血糖水平的影响。(C)与对照处理相比，TDCA（左）和TUDCA（右）处理的小鼠回肠中差异表达基因的火山图。（D）TDCA和TUDCA处理对人源Caco-2细胞中CAR1的蛋白和mRNA表达水平的影响。

In this study, we analyzed changes in serum metabolites of mice on a ketogenic diet and found that serum levels of TDCA and TUDCA were significantly elevated (Figure A) which were related to the reduction in body weight and fasting blood glucose levels (Figure B). Mechanistically, we found that the ketogenic diet significantly reduced the abundance of *Lactobacillus murinus ASF361*, a bacterium encoding bile salt hydrolase (BSH), in the intestinal of mice, which elevated the levels of TDCA and TUDCA. Further, we analyzed the differentially expressed genes in the ileal tissue of Vehicle, TDCA- and TUDCA-treated mice, and the data showed that TDCA- and TUDCA-treatment significantly suppressed Car1 expression in the ileum (Figure C). Meanwhile, we observed the significant inhibition of CAR1 expression under TDCA and TUDCA treatment in the Caco-2 cell level (Figure D). In addition, we examined the expression of genes related to Farnesoid X receptor signaling in intestinal tissues and bile acid synthesis genes in liver tissues, and found that KD did not affect body weight and blood glucose levels through these pathways. Taken together, our results suggested that the ketogenic diet can reduce energy absorption and lead to weight loss by reducing the abundance of *Lactobacillus muridarum ASF361* or inhibiting BSH activity in the intestine, increasing circulating levels of TDCA and TUDCA, and thereby inhibiting the expression of intestinal *Car1*. The data of this study support that TDCA and TUDCA are two promising candidates for the treatment of obesity and its complications in addition to the ketogenic diet.

在本文中，我们检测了生酮饮食小鼠血清代谢物的变化，发现血清中TDCA和TUDCA的水平显著升高（图A），并介导了常规饮食小鼠的体重和空腹血糖水平的降低（图B）。机制上，我们发现生酮饮食显著的降低了小鼠肠道中编码胆汁盐水解酶（BSH）的细菌Lactobacillus murinus ASF361的丰度，从而升高了TDCA和TUDCA的水平。进一步的，我们分析了TDCA和TUDCA处理后的小鼠回肠中的差异表达基因，数据显示TDCA和TUDCA处理显著抑制了回肠中Car1的表达，同时我们在Caco-2细胞水平上也观察到TDCA和TUDCA处理显著抑制了CAR1的表达（图C）。此外，我们通过对肠道组织中Farnesoid X receptor信号相关基因和肝脏组织中的胆汁酸合成基因的表达情况的检测，排除了KD通过这些途径影响体重和血糖的可能。综上，我们的研究结果表明生酮饮食能够通过减少小鼠肠道中鼠乳杆菌ASF361的丰度或抑制BSH活性，增加TDCA和TUDCA的循环水平，进而抑制肠道Car1的表达来介导能量吸收的减少和体重的减轻。本研究的数据支持TDCA和TUDCA是除生酮饮食外治疗肥胖及其并发症的两种有前途的候选药物。

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LC-MS和激光共聚焦分析蛋白磷酸化和亚细胞定位

复旦大学李琳教授课题组于*Developmental Cell*杂志上发表了题为“**Dephosphorylation of bZIP59 by PP2A ensures appropriate shade avoidance response in *Arabidopsis***”的研究论文（https://doi.org/10.1016/j.devcel.2024.10.014）。在研究过程中，李琳教授课题组利用液质联用仪-exploris480进行label-free定量磷酸组分析，结果显示bZIP59-S18位点的磷酸化水平在荫影处理中下降；此外，还利用Leica全光谱多光子荧光寿命成像系统检测bZIP59蛋白在植物体内的亚细胞定位，发现bZIP59受荫影诱导入核。该工作揭示了转录因子bZIP59在避荫反应中的快速响应，为避荫反应的精细化调控提供了证据。

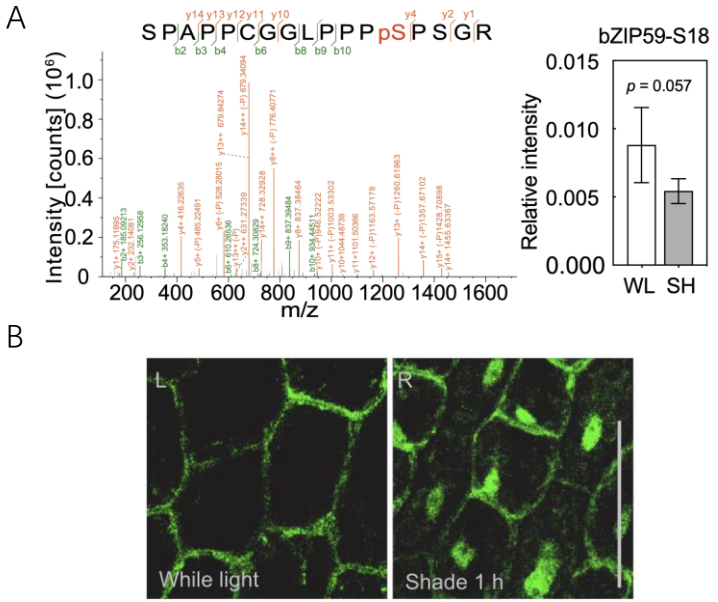

图1.荫影处理诱导转录因子bZIP59去磷酸化和入核；（A）LC-MS/MS定量检测白光（While light，WL）和荫影（Shade，SH）处理下bZIP59-S18位点磷酸化变化；（B）激光共聚焦检测白光和荫影处理下bZIP59-GFP蛋白的亚细胞定位。

Changes in light quality and quantity experienced by many shade-intolerant plants grown in close proximity lead to transcriptional reprogramming and shade avoidance syndrome (SAS). Despite the importance of phosphorylation-dependent signaling in cellular physiology, phosphorylation events during SAS are largely unknown. Here, we examined shade-regulated phosphorylation events in Arabidopsis using quantitative phosphoproteomics. We confirmed shade-induced dephosphorylation of bZIP59, a basic region/leucine zipper motif (bZIP) transcription factor. Shade treatment promotes the nuclear localization of bZIP59, which can be mimicked by mutation of the phosphorylation sites on bZIP59.

四极杆串联静电场轨道阱质谱仪
Thermo Orbitrap Exploris480
负责老师：黄林
仪器位置：D109
电话：31246731

全光谱多光子荧光寿命成像系统
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磷酸化修饰是植物快速响应环境变化的普遍机制。在高密度种植带来的荫影信号中，位于光敏色素下游的转录因子光敏色素结合蛋白（Phytochrome-Interacting Factor）PIF7能够通过磷酸化修饰快速响应光质的变化。除此之外，我们对荫影早期的磷酸化调控还知之甚少。实验室前期通过TMT标记的定量磷酸组学分析，鉴定到一簇属于Group I亚家族的碱性亮氨酸拉链（basic leucine zippers，bZIP）转录因子磷酸化水平在荫影中发生下降。我们利用国重平台液质联用仪-exploris480进行label-free定量磷酸组检测，发现bZIP59-S18磷酸化水平下降，进一步验证了bZIP59在荫影中的去磷酸化。为了探究荫影对bZIP59的影响，我们还利用Leica全光谱多光子荧光寿命成像系统检测其亚细胞定位，发现白光下bZIP59主要分布在细胞质中，荫影能够显著诱导其在细胞核内积累。

供稿人:李琳课题组　撰稿人:李烽全　编辑:刘鹤华

超分辨共聚焦显微镜应用于肥胖和糖尿病的中枢机制研究

复旦大学刘铁民教授课题组于*Cell Metabolism*杂志上发表题为“Region-specific transcriptomic responses to obesity and diabetes in macaque hypothalamus”的文章 (Lei, Ying et al. Tiemin. Liu, *Cell metabolism*. 2024;36(2):438-453.e6. https://www.sciencedirect.com/science/article/pii/S1550413124000032)。在研究过程中，刘教授课题组利用国重平台的Zeiss LSM880 with Airyscan 超分辨共聚焦显微镜对人脑组织切片进行荧光成像、拍摄。发现室旁核中的Oxytocin (OXT)神经元显示出免疫反应显著减少。推测糖尿病中的高血糖可能通过负反馈方式调节室旁核神经元，反过来调节自主和神经内分泌输出以正常化葡萄糖调节。本研究的发现支持大脑葡萄糖感应和神经调节缺陷在糖尿病高血糖中起着关键作用的观点。高分辨率的图片为我们的研究结果提供了强有力的证据。

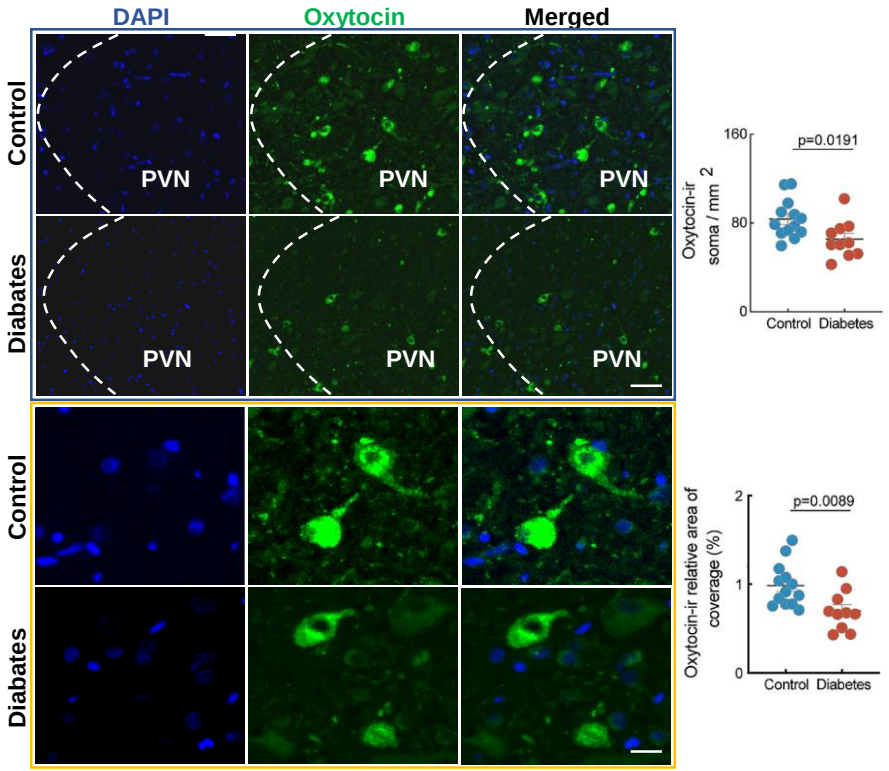

图1 Oxytocin在对照组和糖尿病患者PVN中的免疫荧光染色结果（左图）。对照组和糖尿病患者的PVN Oxytocin覆盖的大小和相对面积统计结果（右图）。

Figure 1 Immunofluorescent of OXT in the PVN of the control and diabetic patients. (left) The size and relative area of coverage of the PVN OXT statistical result in control and diabetic patients. (right)

Oxytocin (OXT), a hypothalamic neuropeptide, plays a pivotal role in the paraventricular nucleus (PVN), modulating energy balance through central signaling pathways. OXT neurons innervate sympathetic premotor neurons in the rostral medullary raphe (rMR), driving brown adipose tissue (BAT) thermogenesis and cardiovascular regulation. In diabetic models, PVN OXT neurons exhibit significant atrophy, characterized by reduced numbers and soma shrinkage. Leveraging the Zeiss LSM880 with Airyscan super-resolution confocal microscope from the National Key Laboratory of Genetic Engineering, we achieved unprecedented imaging precision, elucidating the critical role of PVN glucose-sensing and neuroregulatory impairments in hyperglycemia.

催产素（OXT）是一种由下丘脑分泌的神经肽，特别是在室旁核（PVN）区域发挥关键作用。OXT通过与中枢神经系统的其他信号通路相互作用，影响食物摄入和能量消耗，OXT神经元可以通过神经支配延髓中缝区 (rMR) 的交感前运动神经元以刺激棕色脂肪组织（BAT）生热作用和心血管功能，从而达到调节能量平衡的作用。

Zeiss LSM880 with Airyscan
超分辨共聚焦显微镜

Zeiss LSM710
高感度激光扫描共聚焦显微镜

我们观察到糖尿病模型的动物中，PVN区域OXT神经元发生了明显的数量减少和细胞体积变小。借助于遗传工程国家重点实验室的平台的 Zeiss LSM880 with Airyscan 超分辨共聚焦显微镜无与伦比的成像性能，将分辨率和图像质量大大提升，极大助力了大脑葡萄糖感应和神经调节缺陷在糖尿病高血糖中关键作用的发现。

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流式细胞仪分选单细胞双重乳液PCR液滴

复旦大学生命科学学院张经纬课题组于*Analytical Chemistry*杂志上发表题为“**DEPICT-seq: Single-Cell Transcriptomic Analysis of Rare Cell Subsets Isolated via Nucleic Acid Cytometry**”的文章 (K. Bao. et al. J. Zhang, *Anal Chem.* 2024;96(41):16236-16243.)。在研究过程中，研究人员利用国重平台的**Sony SH800s多功能全自动流式细胞分选仪**和**Olympus New IX73 倒置荧光显微镜**，建立了一种基于双重乳液PCR的核酸靶向细胞亚群单细胞转录组分析方法，称为DEPICT-seq。 DEPICT-seq方法通过整合固定细胞原位生物素化逆转录和双重乳液PCR以识别细胞内部核酸靶标，并利用Sony SH800s对含有阳性/阴性荧光信号的双重乳液液滴进行分选。进一步利用链霉亲和素磁珠回收分选液滴内的生物素化cDNA，并利用优化后的全长转录组扩增方法扩增单细胞转录组文库，为核酸靶向细胞亚群单细胞转录组信息获取提供了一种简单易行的途径。

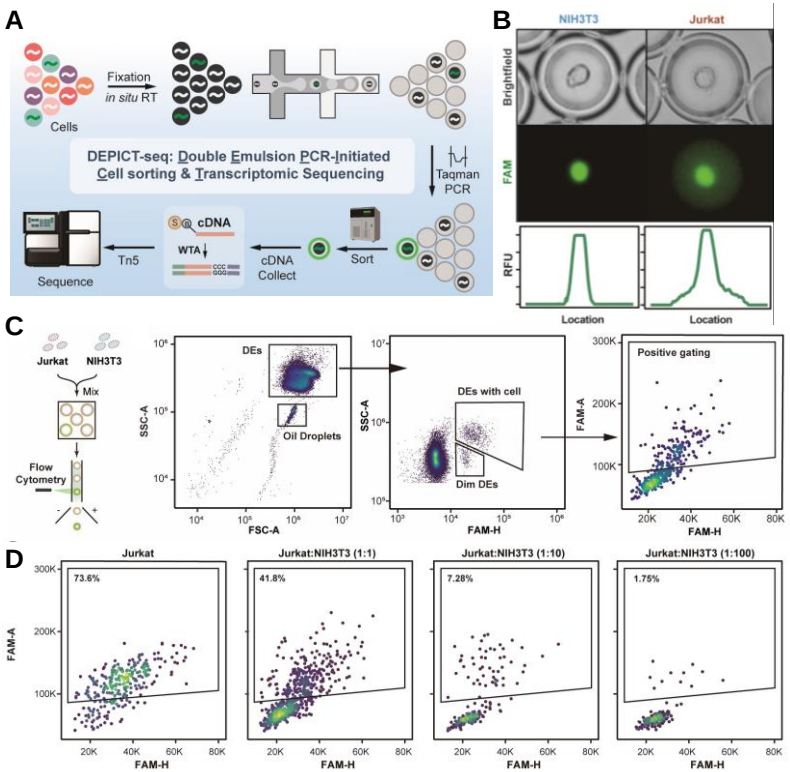

图1流式细胞仪分选单细胞双重乳液PCR液滴。(A)DEPICT-seq技术路线示意图；（B）阳性液滴和阴性液滴经过PCR后呈现出不同的荧光特征；(C) 流式细胞仪分选双重乳液PCR液滴的圈门策略；（D）人鼠细胞混合样品流式分析显示双重乳液PCR可准确识别不同样本中的阴性阳性细胞比例。

The ability to deeply investigate specific rare cell populations is essential for understanding tissue physiology and pathology across various biological domains. With the rapid advancement of single-cell RNA sequencing (scRNA-seq), many newly discovered cell subtypes are defined by their transcriptomic markers. However, our capacity to retrieve and analyze cells based on nucleic acid markers remains limited.

Here, we present Double Emulsion PCR-Initiated Cell sorting and Transcriptomic Sequencing (DEPICT-seq), a high-throughput droplet nucleic acid cytometry method that integrates batch cell fixation for cellular information preservation, double emulsion digital PCR-based cell sorting to target nucleic acid markers of interest, and in-depth full-length transcriptomic analyses at single-cell resolution.

Leveraging the imaging capabilities of the Olympus New IX73 microscope, we performed flow cytometry on post-PCR double emulsions (DEs) containing Jurkat cells, NIH3T3 cells, or their mixtures, using the Sony SH800s cell sorter. During sorting, gating was based on FAM-H versus FAM-A signals to select DEs with fluorescence distributed uniformly throughout the droplet volume. Subsequent microscopic examination of individual wells confirmed the reliable sorting of positive DE droplets.

In conclusion, DEPICT-seq represents a novel and powerful approach for investigating biologically significant cell subpopulations, offering unprecedented insights into cellular diversity and function.

Sony SH800s
多功能全自动流式细胞分选仪
负责老师: 余垚

Olympus New IX73
倒置荧光显微镜
负责老师: 杨建鸽

深入研究特定稀有细胞群体的能力对于理解不同生物领域中的组织生理和病理至关重要。随着单细胞测序的快速发展，许多新发现的细胞亚型通过其转录组标志物得以定义。然而，基于核酸标志物检索和分析细胞的能力上仍然有限。DEPICT-seq结合了批量细胞固定和原位逆转录以保留细胞信息，利用双重乳液数字PCR定位和分选具有核酸靶标的单个细胞，并通过后续cDNA回收和建库实现单细胞水平全长转录组的深入分析。

为了验证方法的可靠性，进行了基于不同Jurkat 细胞、NIH3T3 细胞比例的人工混合物的实验验证。借助 Olympus New IX73 荧光显微镜的成像能力明确了阴阳液滴荧光信号特征，并利用Sony SH800s对PCR后的双重乳液进行了批量分析、分选。在分选过程中，根据 FAM-H 与 FAM-A 信号设定门限，选择荧光均匀分布于整个液滴体积的阳性双乳液。随后，通过荧光显微镜对各孔内的双重乳液进行观察，验证了Sony SH800s对阳性双乳液的可靠分选。总之，DEPICT-seq 提供了一种新颖且强大的方法，用于研究具有生物学意义的细胞亚群体，为探索细胞多样性及其功能提供了前所未有的洞察力。

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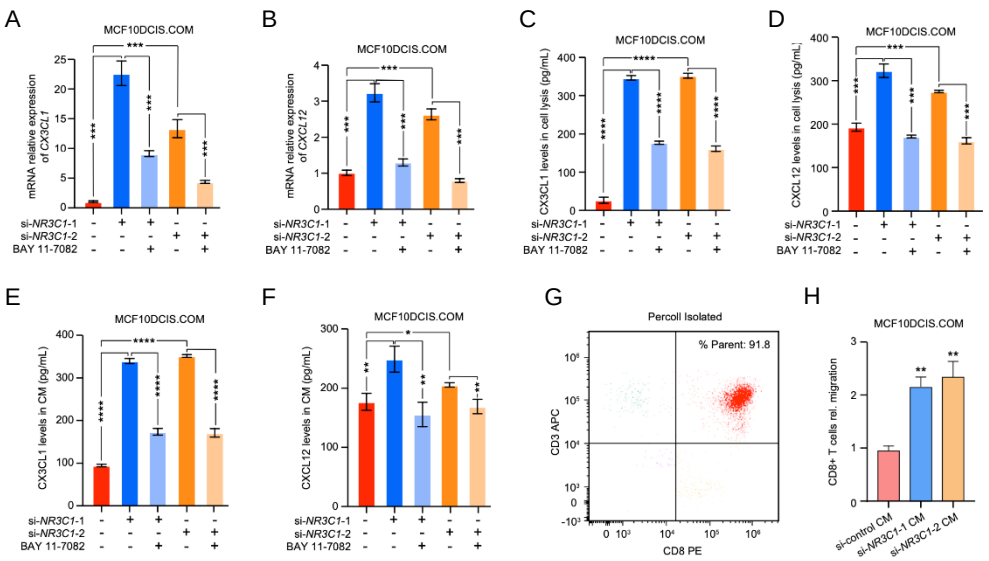
超速流式细胞分选仪在乳腺癌多组学研究中的应用

复旦大学丁琛教授课题组于*Advanced Science*杂志上发表题为“**Proteogenomic Landscape of Breast Ductal Carcinoma Reveals Tumor Progression Characteristics and Therapeutic Targets**”的文章 (G. Xu, et al. C. Ding, Adv Sci. 2024 Oct 17:e2401041. doi: 10.1002/advs.20241041)。在研究过程中，丁琛教授课题组利用超速流式细胞分选仪分离和测定CD8+ T细胞，并和不同条件的细胞共培养，以确定敲低乳腺癌细胞中NR3C1的表达，可以促进CX3CL1和CXCL12的转录水平和蛋白表达水平，从而招募更多的CD8+ T细胞。最后结果验证了我们的假设， 在乳腺癌中，NR3C1的表达下调了CX3CL1和CXCL12的表达，导致其对CD8+ T细胞的趋化性降低，因此导致乳腺癌中的免疫逃逸。



A-D. 与野生型细胞相比，CX3CL1和CXCL12的转录水平和蛋白表达水平在NR3C1敲低的细胞中显著上调。
E-F. CX3CL1和CXCL12的蛋白表达水平在NR3C1敲低组的培养基中显著上调，使用NF-κB抑制剂BAY 11-7082处理后，表达水平显著下调。**G.** 从乳腺癌患者的PBMC中分离出CD8+ T细胞，并测定纯度。
H. 与对照组相比，在NR3C1敲低的细胞的培养基中可以吸引更多的CD8+ T细胞。

Through multigroup analysis, the authors found that the copy number of NR3C1 was variable and had cis effect, which made its protein expression significantly up-regulated in breast cancer. Therefore, the author further verified the downstream pathway of NR3C1 protein expression in breast cancer. First, the author constructed a breast cancer cell line with NR3C1 knockdown. Compared with wild-type cells, the transcription and protein expression levels of CX3CL1 and CXCL12 were significantly upregulated in NR3C1 knockdown cells. The protein expression levels of CX3CL1 and CXCL12 were significantly upregulated in the NR3C1 knockdown group culture medium, and significantly downregulated after treatment with NF - κ B inhibitor BAY 11-7082. CD8+T cells were further isolated from PBMC of breast cancer patients by ultrafast flow cytometry, and their purity was determined. Compared with the control group, the culture medium of NR3C1 knockdown cells can attract more CD8+T cells.

BD FACS Calibur
流式细胞分析仪
负责老师：沈素芹

MoFlo-XDP
超速流式细胞分选仪
负责老师：刘龙江

通过多组学分析，作者发现*NR3C1*的拷贝数存在变异，且具有*cis*作用，使得其蛋白表达在乳腺癌中显著上调。因此，作者进一步验证了NR3C1蛋白表达在乳腺癌中影响的下游途径。首先，作者构建了NR3C1敲低的乳腺癌细胞系。与野生型细胞相比，CX3CL1和CXCL12的转录水平和蛋白表达水平在NR3C1敲低的细胞中显著上调。CX3CL1和CXCL12的蛋白表达水平在NR3C1敲低组的培养基中显著上调，使用NF-κB抑制剂BAY 11-7082处理后，表达水平显著下调。作者进一步利用超速流式细胞分选仪，从乳腺癌患者的PBMC中分离出CD8+ T细胞，并测定纯度。与对照组相比，在NR3C1敲低的细胞的培养基中可以吸引更多的CD8+ T细胞。

这些结果验证了假设，即在乳腺癌中，NR3C1的表达下调了CX3CL1和CXCL12的表达，导致其对CD8+ T细胞的趋化性降低，因此导致乳腺癌中的免疫逃逸。

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