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·基础研究·

单细胞组学解析ACVR1在成牙本质细胞分化中的作用

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【摘要】　目的　基于单细胞测序探究活化蛋白A受体1型（ACVR1）在成牙本质细胞分化及牙本质形成中的影响。**方法**　利用GEO数据库健康野生型成年小鼠切牙单细胞数据（GSE146123）进行ACVR1与成牙相关转录因子的相关性分析。本实验已获得所在单位的实验动物伦理委员会批准。利用Cre-LoxP系统构建牙源性间充质细胞中ACVR1条件性敲除小鼠（实验组：Osterix-Cre；ACVR1$^{fx/-}$，对照组：Osterix-Cre；ACVR1$^{fx/+}$）。分别提取3周龄小鼠切牙牙髓组织制备单细胞悬液，进行10×Genomics单细胞转录组测序。采用Seurat流程进行质控、标准化、降维及Harmony批次效应校正，通过UMAP降维可视化与细胞亚群鉴定；运用Monocle进行拟时序分析推断细胞分化轨迹，单细胞数据层面分析与基因ACVR1呈正相关的转录因子的变化。通过免疫荧光染色验证关键转录因子Osterix及成牙本质细胞特异性蛋白牙本质涎磷蛋白（DSPP）的定位与表达变化。**结果**　Sp7/ Osterix在小鼠切牙间充质细胞群（GSE146123）中广泛表达，利用Osterix-Cre，可有效敲除间充质细胞中的ACVR1。ACVR1与成牙关键基因相关性分析显示：ACVR1与Msh同源框1（Msx1）、Msh同源框2（Msx2）、Sp7转录因子（Sp7）等成牙关键基因呈现正相关性。绘制对照组与实验组切牙牙髓组织单细胞图谱，并发现实验组前成牙本质细胞数量比例增加最明显，同时该亚群与成牙本质细胞分化和极化相关的关键基因的表达均下调。随后对所有间充质细胞亚群进行差异基因富集分析发现，大部分与牙本质形成相关的基因表达均下调。最后通过单细胞差异基因分析，基因随拟时序表达分析及免疫荧光染色验证，实验组中成牙本质细胞中Sp7和DSPP的表达明显下调。**结论**　ACVR1基因可能通过Sp7、Msx1、Msx2等转录因子促进成牙本质细胞分化进而影响牙本质的形成。

【关键词】　成牙本质细胞分化；　活化蛋白A受体1型；　Msx1转录因子；　Msx2转录因子；　Sp7转录因子；　牙源性间充质细胞；　单细胞测序；
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Single-cell omics reveals the role of ACVR1 in odontoblastic differentiation

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【Abstract】　Objective　To investigate the impact of activin receptor type-1 (ACVR1) on odontoblastic differentiation and dentin formation based on single-cell RNA sequencing. **Methods**　A correlation analysis of ACVR1 with

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odontogenesis-related transcription factors was performed using single-cell data from healthy wild-type adult mouse incisors obtained from the Gene Expression Omnibus database (GSE146123). This experiment was approved by the Institutional Animal Care and Use Committee of the affiliated institution. Conditional knockout mice with ACVR1 deleted in the dental mesenchyme were generated using the Cre-LoxP system (experimental group: Osterix-Cre; ACVR1$^{fx/-}$, control group: Osterix-Cre; ACVR1$^{fx/+}$). Dental pulp tissue was extracted from the incisors of 3-week-old mice to prepare single-cell suspensions for 10 × Genomics single-cell transcriptome sequencing. Quality control, normalization, dimensionality reduction, and Harmony batch effect correction were performed using the Seurat pipeline, followed by UMAP dimensionality reduction visualization and cell subpopulation identification. Monocle was used for pseudotime analysis to infer cell differentiation trajectories, and changes in transcription factors positively correlated with ACVR1 were analyzed at the single-cell data level. Immunofluorescence staining was employed to validate the localization and expression changes of the key transcription factor Osterix and the odontoblast-specific protein DSPP. **Results**　Sp7/Osterix is widely expressed in the incisor mesenchyme of mice (GSE146123). Using Osterix-Cre, ACVR1 was effectively knocked out in the mesenchyme. Gene correlation analysis between ACVR1 and key odontogenic genes showed positive correlations between ACVR1 and genes such as Msx1, Msx2, and Sp7. Single-cell atlases of incisor dental pulp tissue from "Osterix-Cre; ACVR1$^{fx/-}$" and "Osterix-Cre; ACVR1$^{fx/+}$" mice were then constructed. The most significant increase in the proportion of pre-odontoblasts was in the experimental group, along with downregulated expression of key genes related to odontoblastic differentiation and polarization in this subpopulation. Differential gene enrichment analysis across all mesenchymal subpopulations indicated downregulated expression of most genes associated with dentin formation. Finally, as observed through single-cell differential gene analysis, pseudotime expression analysis, and immunofluorescence validation, the expression of Sp7 and DSPP were significantly downregulated in the experimental group. **Conclusion**　The ACVR1 gene may promote odontoblastic differentiation and subsequently affect dentin formation through transcription factors such as Sp7, Msx1, and Msx2.

【Key words】 odontoblastic differentiation;　activin receptor type-1;　Msx1 transcription factor;　Msx2 transcription factor;　Sp7 transcription factor;　dental mesenchymal cells;　single-cell sequencing;　bioinformatics analysis
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牙本质是构成牙齿的主体结构，对于保护牙髓组织及保持牙齿结构与功能健全具有关键作用[1]。牙本质形成的细胞学基础源于牙髓间充质干细胞向成牙本质细胞谱系的定向分化[2-3]。首先干细胞分化为前成牙本质细胞，随后经细胞极性建立等生物学行为分化为成牙本质细胞，最终成熟的成牙本质细胞合成并分泌包括Ⅰ型胶原（type Ⅰ collagen，Col1）和牙本质涎蛋白（dentin sialoprotein，DSP）、牙本质基质蛋白1（dentin matrix protein，DMP1）等多种细胞外基质成分，后经矿化等过程逐步形成完整的牙本质结构[4-5]。因此，如何调控牙髓干细胞向成牙本质细胞谱系分化的这一生物学过程，对于促进健康牙本质的形成具有关键意义。

多种信号通路协同调控干细胞向成牙本质细胞谱系分化的发育过程，其中骨形态发生蛋白（bone morphogenetic protein，BMP）信号通路在此过程中不可或缺[6-7]。活化蛋白A受体1（activin receptor type-1，ACVR1）属于Ⅰ型BMP受体家族。研究表明，特异性敲除小鼠神经嵴细胞中的ACVR1基因，可导致颅颌面区域出现多种发育畸形[8]，本课题组前期研究发现，利用Osterix-Cre特异性敲除牙源性间充质细胞中的ACVR1，导致小鼠切牙骨样牙本质的形成及成牙本质细胞极性丧失等分化异常[9]。ACVR1如何调控其下游信号影响成牙本质细胞分化，目前尚不清晰。

因此，为深入解析ACVR1在成牙本质细胞分化中的调控功能，本研究利用Osterix-Cre系统构建了牙源性间充质细胞特异性ACVR1基因敲除小鼠模型，并通过单细胞转录组测序技术[4]，系统揭示间充质细胞中关键成牙相关转录因子的动态变化。

1　材料和方法

1.1　试剂与仪器

兔来源的抗小鼠Osterix一抗（ab209484，Abcam，英国），小鼠来源的抗小鼠牙本质涎磷蛋白（dentin sialophosphoprotein，DSPP）一抗（sc-65495，

Santa Cruz,美国),AF488荧光标记的山羊抗小鼠IgG二抗(ab150077,Abcam,英国)和AF647荧光标记的山羊抗小鼠IgG二抗(ab150115,Abcam,英国),兔来源的抗小鼠p-Smad1/5/8一抗(13820,CST,美国)。激光共聚焦显微镜(AXR,Nikon,日本)。牛血清白蛋白(BSA)(北京兰杰柯科技有限公司,中国),石蜡切片机(Leica公司,德国);柠檬酸钠修复液(北京索莱宝科技有限公司,中国)

1.2　数据集分析

本研究部分数据来自GEO(Gene Expression Omnibus)数据库(https://www.ncbi.nlm.nih.gov/geo/),数据集编号:GSE146123。样本来源为成年野生型健康小鼠,共使用78个下颌切牙,总计39只小鼠。本研究使用该数据经过质控筛选后的小鼠切牙间充质细胞群(共1 111个细胞)。

1.3　实验动物与分组

本实验遵守动物研究指南:体内实验报告(The Animal Research: Reporting of In Vivo Experiments,ARRIVE)指南(https://arriveguidelines.org),并且已获得吉林大学基础医学院实验动物伦理委员会批准,批准编号:(2026年)研审第(4)号。采用Cre-Loxp系统构建ACVR1条件性敲除小鼠模型。实验组为Osterix-Cre; $ACVR1^{fx/-}$小鼠,对照组为Osterix-Cre; $ACVR1^{fx/+}$小鼠,所有小鼠品系为C57BL/6J,均源自美国密歇根大学牙学院Yuji Mishina实验室。小鼠体质量15~25 g,雌雄各半,动物性别对结果无影响。实验动物使用许可证号为SYXK(吉)2023-0010,分别取对照组与实验组3周龄小鼠用于实验,每组n≥6,实验动物饲养于吉林大学基础医学院动物房(SPF级)。

1.4　单细胞测序样品准备

基于10X Genomics单细胞测序平台,选取3周龄实验组和对照组小鼠各4只,在体视显微镜下分离下颌切牙,去除周围硬组织后获取牙髓。将牙髓用Dulbecco's磷酸盐缓冲液(DPBS,Biosharp,中国)清洗后,转移至含3 mg/mL胶原酶I(Sigma,美国)与4 mg/mL分散酶(Sigma,美国)的酶消化液中。将样本于37 ℃孵育30 min,每10 min轻微振摇以分散细胞。加入含10% 胎牛血清(FBS)的DPBS终止消化,经70 μm滤网过滤。300 × g离心3 min收集细胞,加入3 mL红细胞裂解液于4 ℃裂解3 min去除红细胞。再次300 × g离心3 min,用含0.1% FBS的1 × DPBS重悬细胞并进行计数。

1.5　单细胞转录组数据处理与分析

1.5.1　数据预处理与质量控制　在R语言环境(版本4.4.3)中使用Seurat软件包(版本5.3.0)对单细胞转录组数据进行了预处理和质量控制。为获得高质量数据,设定了以下细胞筛选标准:①保留每个细胞检测到的基因数(nFeature_RNA)为200~5 000之间的细胞,以过滤掉因RNA含量过低或过高而可能代表死亡细胞、碎片或双细胞/多细胞混合的数据点;②排除线粒体基因表达占比(percent.mt)高于20%的细胞,以去除因细胞膜破损导致RNA显著降解的凋亡或低活性细胞;③移除每个细胞总分子标签数(nCount_RNA)超过30 000的细胞,以规避可能由多细胞液滴或技术假象产生的高测序深度数据;通过上述预处理与质控步骤,本实验构建了一个高质量的表达矩阵,用于后续的细胞分群与深入分析。

1.5.2　数据标准化与整合　为消除测序深度差异,本实验采用NormalizeData函数对UMI计数进行对数归一化(LogNormalize)。随后使用FindVariableFeatures筛选出表达变异度最高的2 000个基因作为高变基因集。为校正技术噪声,通过ScaleData对表达数据进行线性缩放,并在模型中回归每个细胞的线粒体基因占比(percent.mt)与总UMI数(nCount_RNA)的影响。基于缩放后的高变基因表达矩阵进行主成分分析(principal components analysis,PCA),并以前12个主成分的基因载荷、PCA散点图及前两个主成分的热图(基于500个随机细胞)初步评估降维效果与细胞群结构。

1.5.3　细胞聚类、注释与差异表达分析　在完成数据整合与降维后,本实验基于前20个主成分,利用Find Neighbors函数构建细胞近邻图,随后采用FindClusters函数进行无监督聚类(Louvain算法,分辨率设为0.5)。为便于可视化,使用RunUMAP函数进行非线性降维。细胞类型注释通过跨亚群筛选经典标记基因完成,并参考CellMarker数据库及相关文献进行验证。为识别各细胞亚群的特征基因,本实验使用FindAllMarkers函数进行差异表达分析。筛选标准设定为:基因至少在25%的细胞中表达(min.pct = 0.25),且保留表达差异倍数对数化值(avg_log2FC)绝对值大于0.5、校正后P值(P_val_adj)小于0.05的基因作为显著标志物。结果以表格形式导出,并选取代表性基因通过Feature Plot及vPilot进行可视化展示。

1.5.4　基因相关性分析　通过文献与数据库

（PubMed、GeneCards）筛选已知调控成牙本质分化的关键转录因子，后基于GSE146123小鼠间充质单细胞数据，使用Spearman相关系数分析ACVR1与每个转录因子表达的相关性。

1.5.5　细胞轨迹推断分析　为探究细胞分化动态，本实验使用Monocle2软件包对间充质细胞亚群进行拟时序分析。首先，将Seurat对象中提取的RNA计数矩阵及相关细胞元数据转换为Monocle所需CellDatase对象，并指定使用负二项分布（negbinomial.size）作为表达量建模分布。随后，对数据进行尺寸因子与离散度估计，并基于基因表达均值（≥0.1）与经验离散度高于拟合离散度的条件，筛选用于轨迹构建的排序基因集。采用DDRTree方法进行非线性降维，进而通过order Cells函数构建细胞发育轨迹并进行拟时序排序。为明确轨迹起始，本实验根据Sfrp2和Smoc2等早期牙间充质细胞标记基因的高表达模式指定根状态，从而获得具有明确生物学指向的拟时序排列。结果通过plot_cell_trajectory函数可视化，分别按细胞聚类、拟时序值及轨迹状态进行着色，以展示细胞沿轨迹的分布。

1.6　小鼠切牙样本的制备与免疫荧光染色法染色

分别采集对照组与实验组小鼠的切牙，置于4%多聚甲醛溶液中，于4 ℃下固定过夜，固定液需完全浸没组织。固定后，样本以流动自来水冲洗30 min，去除残留固定液；随后转入10% EDTA脱钙液中，在摇床上以约60 rpm持续缓慢振荡，脱钙3周，每2 d更换一次脱钙液。以组织质地明显变软、切片时无阻滞感为脱钙完全的标准。脱钙完成后，样本经梯度浓度乙醇（70%、80%、95%、100%）逐级脱水、二甲苯透明、熔融石蜡浸渗与包埋，对切牙组织进行连续切片，厚度3 μm。所得切片于4 ℃保存，以维持抗原免疫活性。石蜡切片（3 μm）经常规脱蜡及水化处理，采用EDTA高压修复法进行抗原修复，随后以PBS洗涤3次，每次5 min，并使用3% H_2O_2室温孵育10 min以阻断内源性过氧化物酶活性。再次PBS洗涤后，使用5% BSA室温封闭1 h。滴加兔来源的抗Osterix一抗（Abcam，#ab209484，稀释比例1∶200），小鼠来源的抗DSPP（Santa Cruz，#sc-65495，稀释比例1∶200）一抗，4 ℃孵育过夜。次日复温后，PBS洗涤，滴加AF488荧光标记的山羊抗小鼠IgG二抗（Abcam，#ab150077，稀释比例1∶500）和AF647荧光标记的山羊抗小鼠IgG二抗（Abcam，#ab150115，稀释比例1∶500）；滴加兔来源的抗小鼠p-Smad1/5/8一抗（CST，#13820，稀释比例1∶400），滴加AF488荧光标记的山羊抗小鼠IgG二抗（Abcam，#ab150077，稀释比例1∶500）室温避光孵育2 h。经PBS充分洗涤后，滴加DAPI溶液复染细胞核，封片后于共聚焦显微镜下观察并采集图像。

1.7　统计学分析

单细胞水平基因表达的统计比较中，为分析目标基因在不同分组间的表达差异，本实验首先从校正后的数据中提取表达矩阵，并计算各组的均值与标准差。采用非参数统计方法（Wilcoxon秩和检验）对两组间基因表达水平进行差异分析。组织学定量采用方差分析（ANOVA）进行统计学分析，以$P < 0.05$为差异具有统计学意义。数据比较及作图分析使用Excel和GraphPad Prism（10.6.0）软件完成。

2　结　果

2.1　间充质细胞中Sp7/Osterix的表达

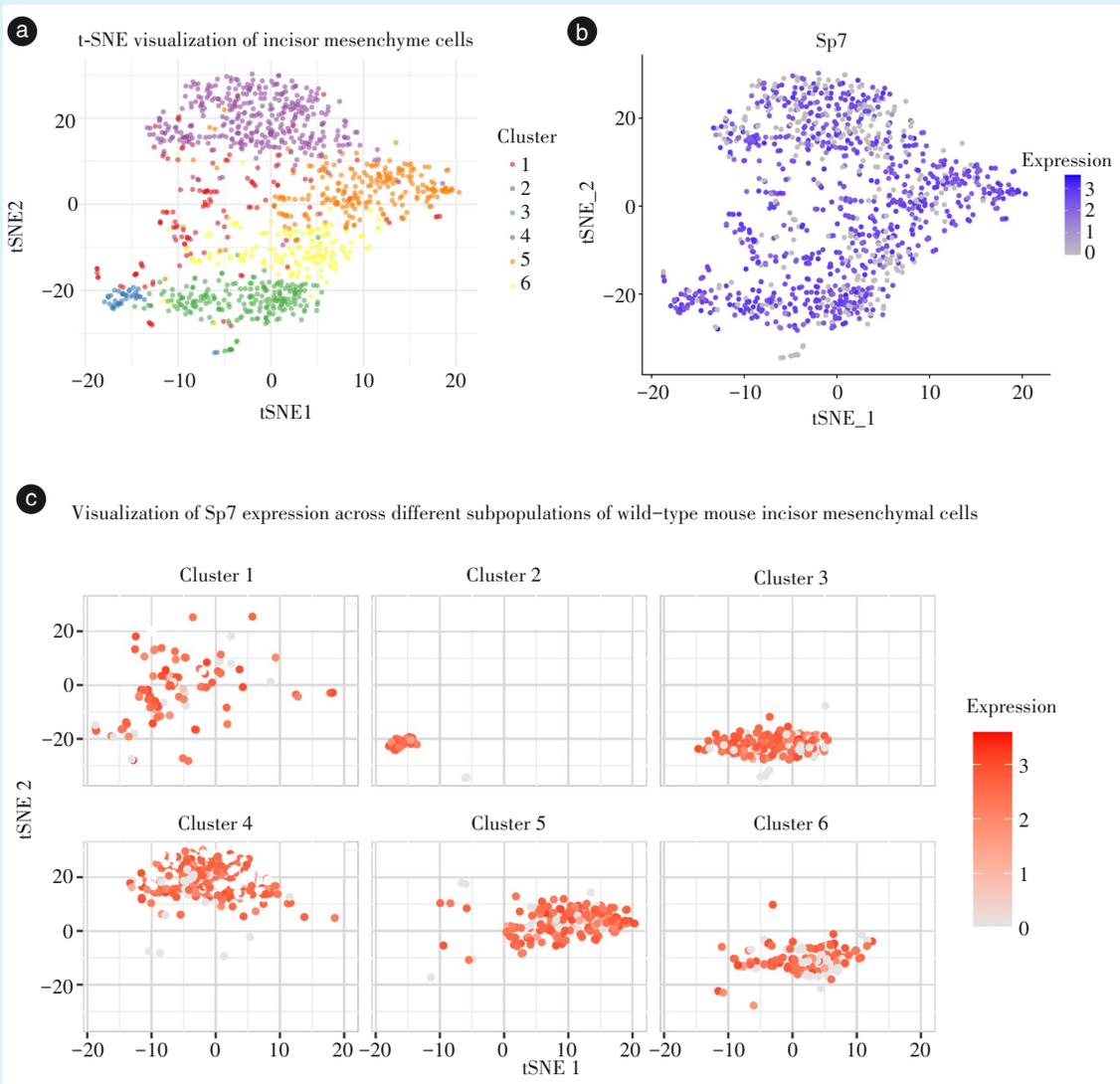
基于数据集GSE146123在单细胞转录组水平发现，野生型小鼠切牙间充质细胞群可大致分为6个亚群，且Sp7/Osterix在各亚群中广泛表达（图1），因此应用Sp7-cre可在小鼠切牙间充质细胞中广泛敲除基因ACVR1。

2.2　ACVR1与成牙关键基因的相关性分析

为了探究ACVR1是否参与调控成牙本质细胞分化及牙本质形成，本实验对野生型小鼠切牙间充质的单细胞转录组数据（GSE146123）进行了分析。首先，相关性分析显示，ACVR1的表达与Msx1、Msx2、Sp7及Dlx6等多个牙发育关键转录因子呈正相关（$P < 0.05$）（图2a）。随后，本实验筛选出25个与ACVR1相关且已知参与牙本质形成或成牙本质细胞分化的基因（$P < 0.05$）（图2b）。值得注意的是，其中包含Sp7——该转录因子是调控成牙本质细胞分化及牙本质生成的核心因子，其与ACVR1的正相关关系提示，ACVR1可能通过直接影响Sp7的表达来调控上述过程（图2c）。此外，通路富集分析表明，这些ACVR1相关基因富集于能够直接或间接调控牙本质形成的信号通路中（$P < 0.05$）（图2d）。

2.3　Osterix-Cre；ACVR1条件性基因敲除小鼠模型间充质单细胞图谱

成年小鼠切牙中成牙本质细胞已分化成熟，其相关性分析可为ACVR1的功能提供广谱预测



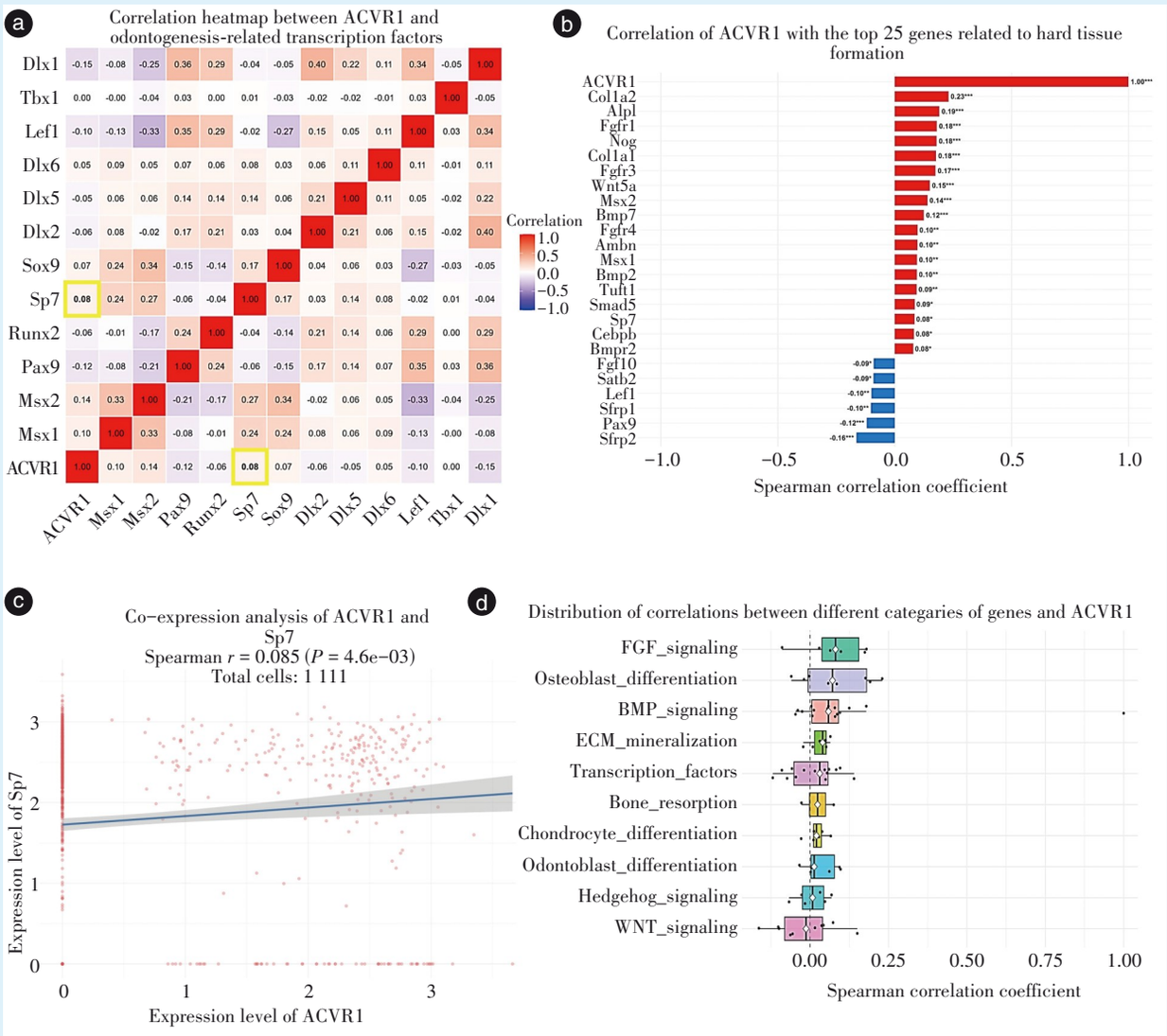
The data for this study was derived from the Gene Expression Omnibus (GEO) database (https://www. ncbi. nlm. nih. gov/geo/; dataset ID: GSE146123), *n* = 39 mice (1 111 cells). a: t-distributed stochastic neighbor embedding (t-SNE) analysis identified six distinct subpopulations within the mouse incisor mesenchymal cells; b-c: Sp7 was broadly expressed across all mesenchymal subpopulations in the mouse incisors. The intensity of red color represents the level of Sp7 expression

Figure 1　Expression pattern of Sp7 in the mesenchymal cells of the adult mouse incisors

图1　Sp7在成年小鼠切牙间充质细胞中的表达情况

依据；而3周龄小鼠则是观察成牙本质细胞分化障碍的最佳窗口期。因此本实验分别构建了3周龄基因条件敲除小鼠“Osterix-Cre；$ACVR1^{fx/-}$”（实验组）与“Osterix-Cre；$ACVR1^{fx/+}$”（对照组）的切牙牙髓组织单细胞转录组图谱（图3a-3c）。免疫荧光染色及UMAP可视化显示，ACVR1主要在间充质细胞群中表达，且实验组间充质细胞中ACVR1的表达较对照组显著下调（图3d-3e）。

进一步对间充质细胞进行亚群分析，共鉴定出10个不同的细胞亚群（图4a-4b）。细胞比例分析表明，实验组中前成牙本质细胞亚群的占比增加最为显著（图4c）。差异基因表达分析显示，该亚群中与成牙本质细胞分化相关的基因表达谱发生明显改变（图4d）。进一步分析发现，实验组前成牙本质细胞中调控成牙本质细胞分化与细胞极化的关键基因（包括Sp7、细胞分裂周期蛋白42（cell division cycle 42，Cdc42）等）表达均显著下调（图4e）。此外，Sp7、Msx1、Msx2等核心基因的变化趋势在两者中保持一致，进一步验证了结果一中预测的可靠性。



The data used in this study comes from the Gene Expression Omnibus database (https://www.ncbi. nlm. NIH. gov/go/), dataset ID: GSE146123, n = 39 mice (1 111 cells). a: correlation analysis was performed between ACVR1 and odontogenic genes using single-cell RNA-seq data (GSE146123) from mouse incisor mesenchymal cells; b: top 25 odontogenic genes showing statistically significant correlations with ACVR1 (*P < 0.05; **P < 0.01; ***P < 0.001); c: co-expression analysis revealed a positive correlation between ACVR1 and Sp7, a key transcription factor for odontoblastic differentiation; d: top 10 signaling pathways closely associated with ACVR1, including pathways implicated in odontoblastic differentiation

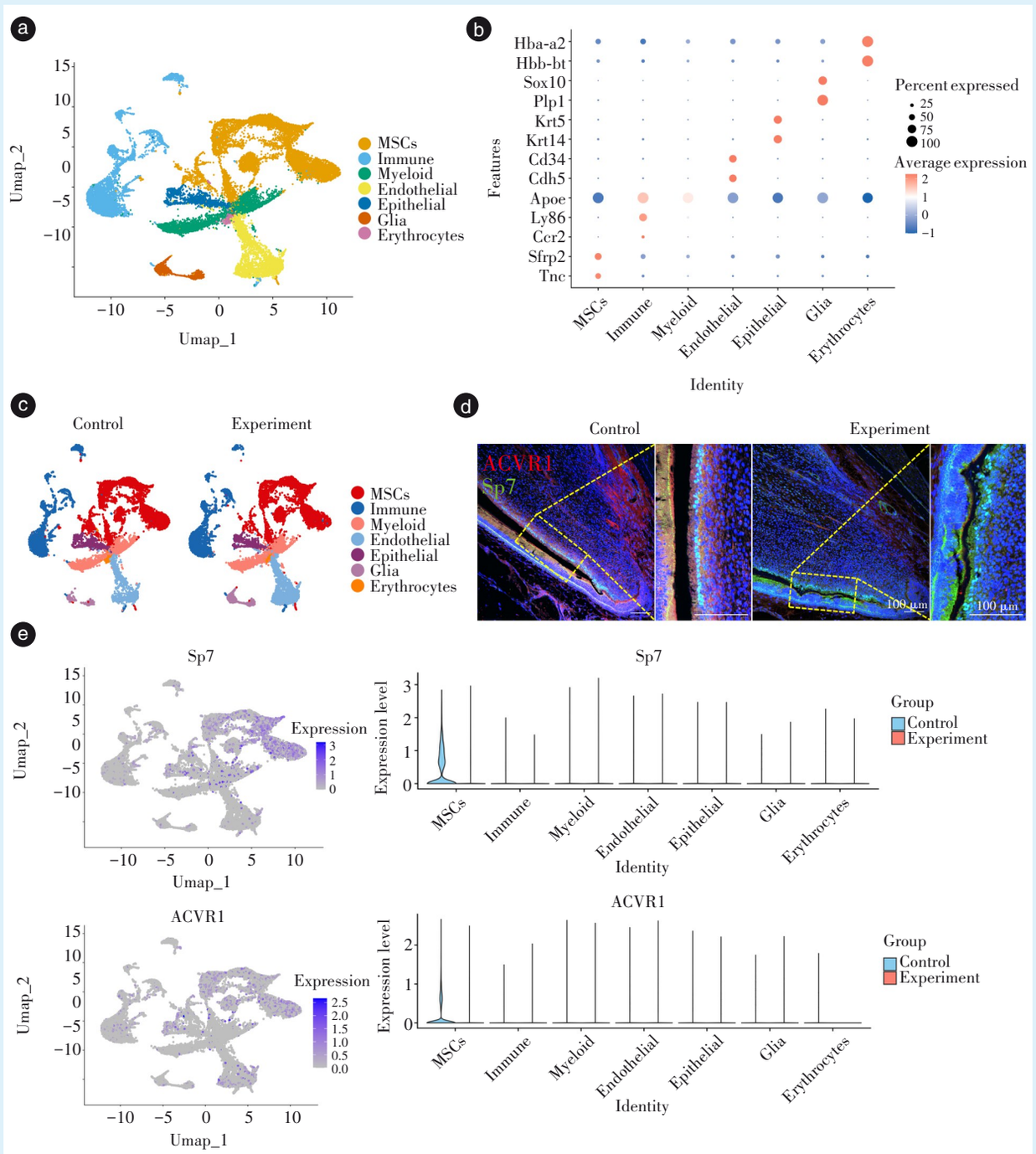
Figure 2　Correlation analysis of ACVR1 with key odontogenic genes

图2　ACVR1与关键成牙基因的相关性分析

2.4　Osterix-Cre；ACVR1条件性基因敲除小鼠模型中间充质细胞成牙关键基因的表达变化

为全面评估间充质细胞的基因表达变化，本实验对实验组与对照组间充质细胞中与牙本质形成相关的调控因子进行了整体表达谱比较（图5a）。通过对上述差异表达基因进行Gene Ontology（CellDatase）富集分析，发现其富集于调控成牙本质细胞分化及牙本质形成的生物学过程与信号通路（图5b）。

进一步，本实验从图5a的结果中选取了与ACVR1呈正相关的关键基因，包括Msx1、性别决定区Y框蛋白9（sex determining region Y-box 9，Sox9）、Twist家族碱性螺旋-环-螺旋转录因子1（twist family bHLH transcription factor 1，Twist1）等，这些转录因子在牙发育过程中对间充质细胞的分化至关重要[10-12]。通过拟时序基因表达轨迹及差异表达分析（图6），发现这些基因在实验组中的表达水平均下调。



This study used the Cre Loxp system to construct an ACVR1 conditional knockout mouse model. Experiment group: Osterix Cre; ACVR1$^{fx/-}$ mice, con trol group: Osterix Cre; ACVR1$^{fx/+}$ mice. All mouse strains are C57BL/6J, sourced from Yuji Mishina Laboratory at the School of Dentistry, University of Michigan, USA. a-c: single-cell transcriptomic atlas of dental pulp tissues from the experiment and control group, n = 4 mice per group; d-e: expression of ACVR1 in the control group *vs*. experiment group, showing significant downregulation in the mesenchymal cells of the experiment group.d: immunofluorescence staining. e: n = 4 mice per group

Figure 3　Mesenchymal single-cell atlas in Osterix-Cre; ACVR1 conditional knockout mice

图3　Osterix-Cre; ACVR1条件性基因敲除小鼠的间充质单细胞图谱

2.5　Osterix-Cre；ACVR1条件性基因敲除小鼠中ACVR1-Sp7-DSPP信号轴破坏

在本研究中，观察到在ACVR1条件性敲除小鼠（实验组）中，Sp7在各类间充质细胞亚群中的表达均呈现一致下调（图7）。免疫荧光染色结果显示，实验组小鼠切牙的间充质区域（包括成牙本质

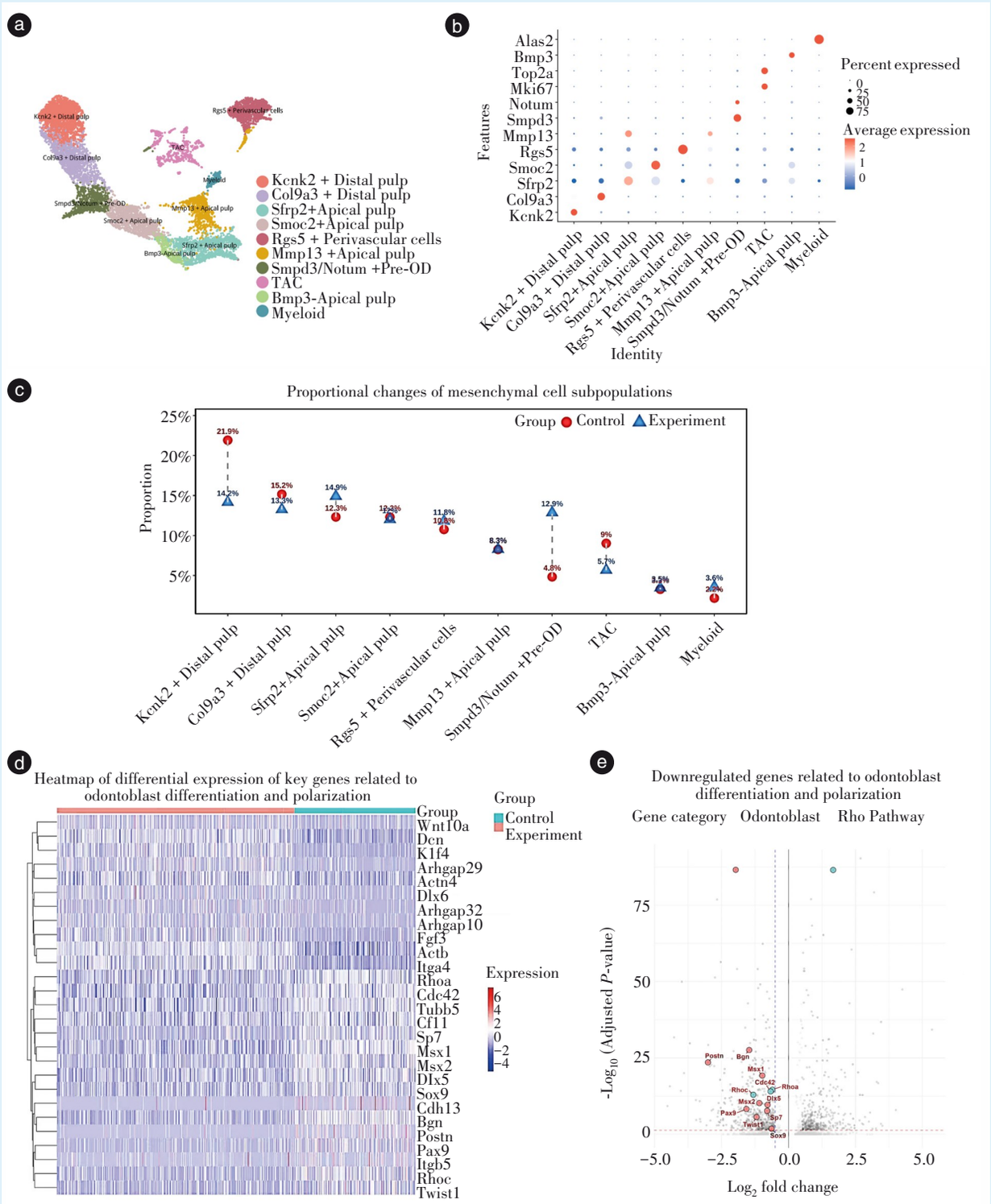


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Figure 4　Mesenchymal single-cell subpopulation dynamics in Osterix-Cre; ACVR1 conditional knockout mice

图4　Osterix-Cre; ACVR1条件性基因敲除小鼠的间充质单细胞亚群动态变化

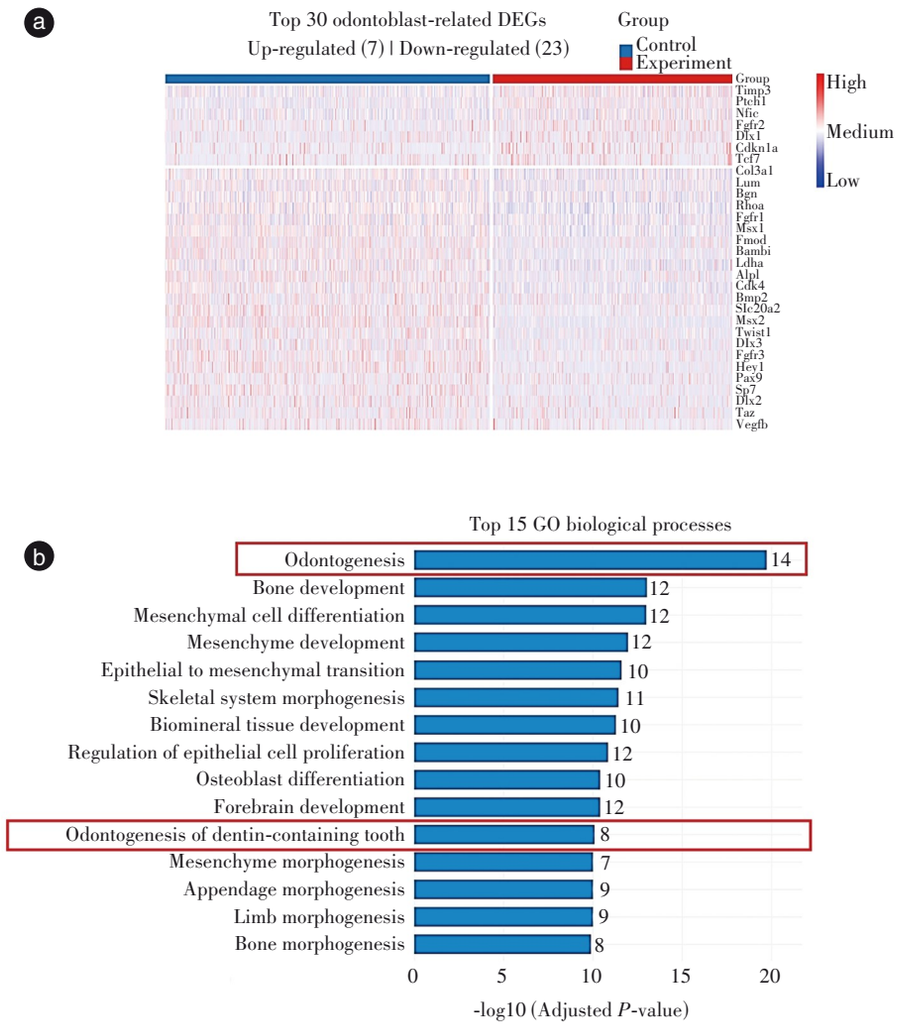

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Figure 5　Differentially expressed genes and Gene Ontology enrichment analysis in the mesenchyme of Osterix-Cre; ACVR1 conditional knockout mice

图5　Osterix-Cre; ACVR1 条件性基因敲除小鼠间充质中的成牙差异表达基因及Gene Ontology富集分析

细胞层)中Osterix蛋白表达显著降低($P < 0.001$),同时其下游关键效应分子DSPP在成牙本质细胞中的表达也明显下降,差异具有统计学意义($P < 0.01$)(图8a-8c);随后探究ACVR1下游Smad信号的变化情况,单细胞水平Smad1/5/8中Smad1无明显变化、Smad5、Smad8的表达量下降,通过组织学免疫荧光染色发现p-Smad1/5/8的表达下调,差异具有统计学意义($P < 0.001$)(图8d-8f)。这些结果共同提示,ACVR1缺失导致p-Smad1/5/8的表达下降,进而下调转录因子Sp7的表达,影响DSPP等牙本质形成相关基因的表达,最终损害牙本质的正常形成。

3　讨　论

在骨发育过程中,成骨细胞的分化及功能维持离不开Sp7(Osterix)的精准调控[13]。同样在牙发育过程中,转录因子Sp7(Osterix)对成牙本质细胞的分化、成熟及其分泌牙本质基质的功能具有

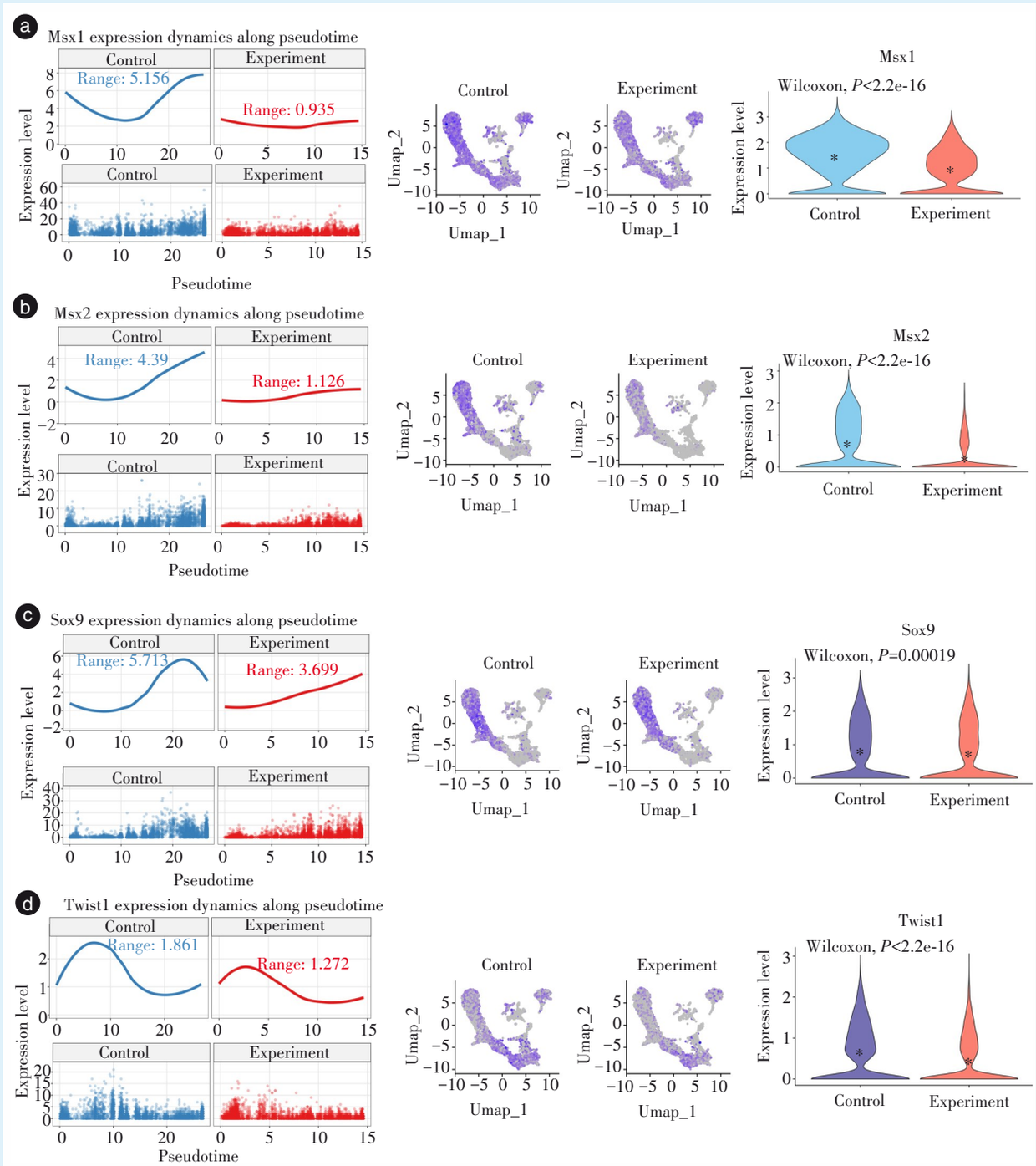

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Figure 6　Odontogenic key gene expression in the mesenchyme of Osterix-Cre; ACVR1 conditional knockout mice

图6　Osterix-Cre; ACVR1条件性基因敲除小鼠间充质中的成牙关键基因表达变化

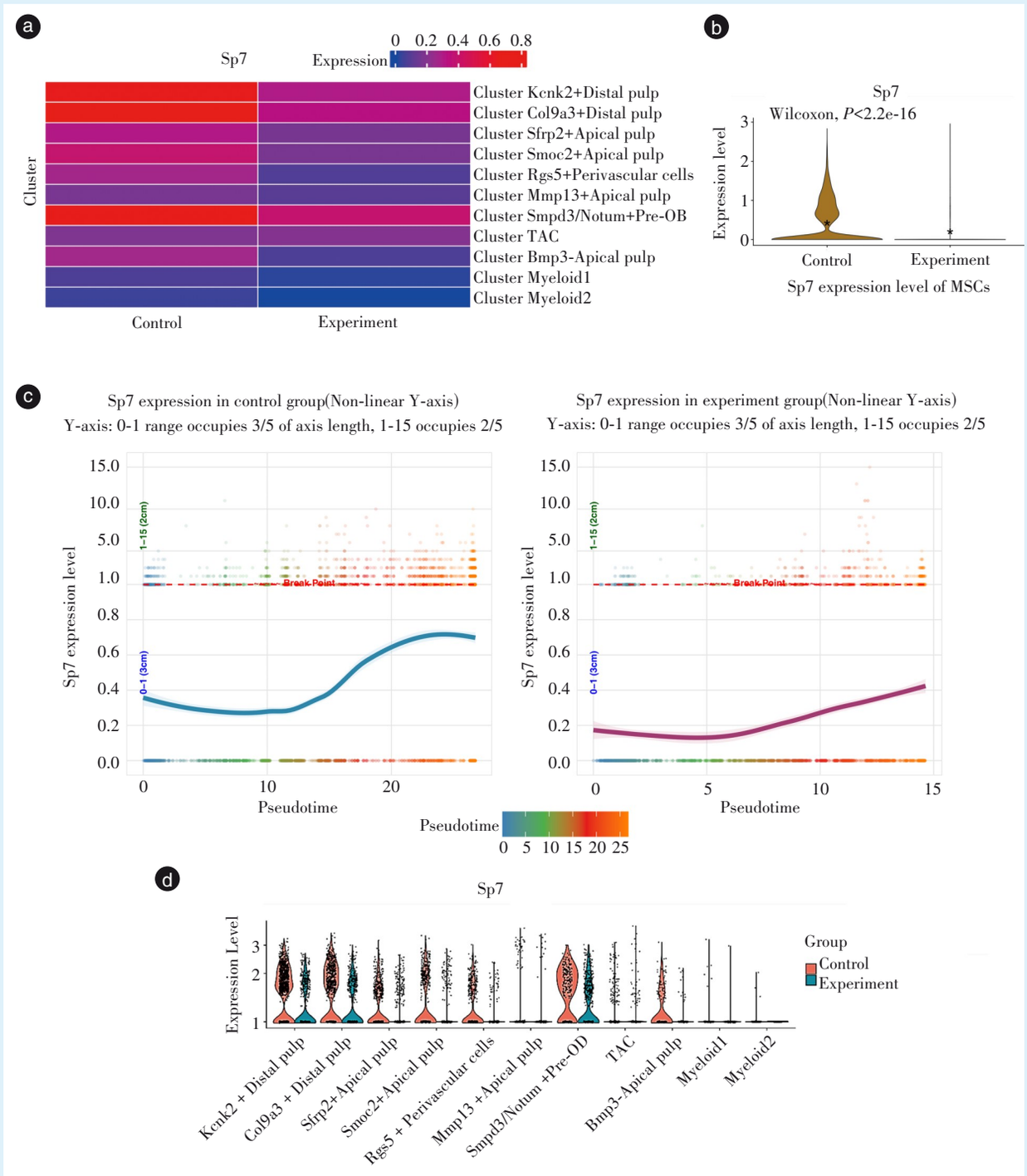

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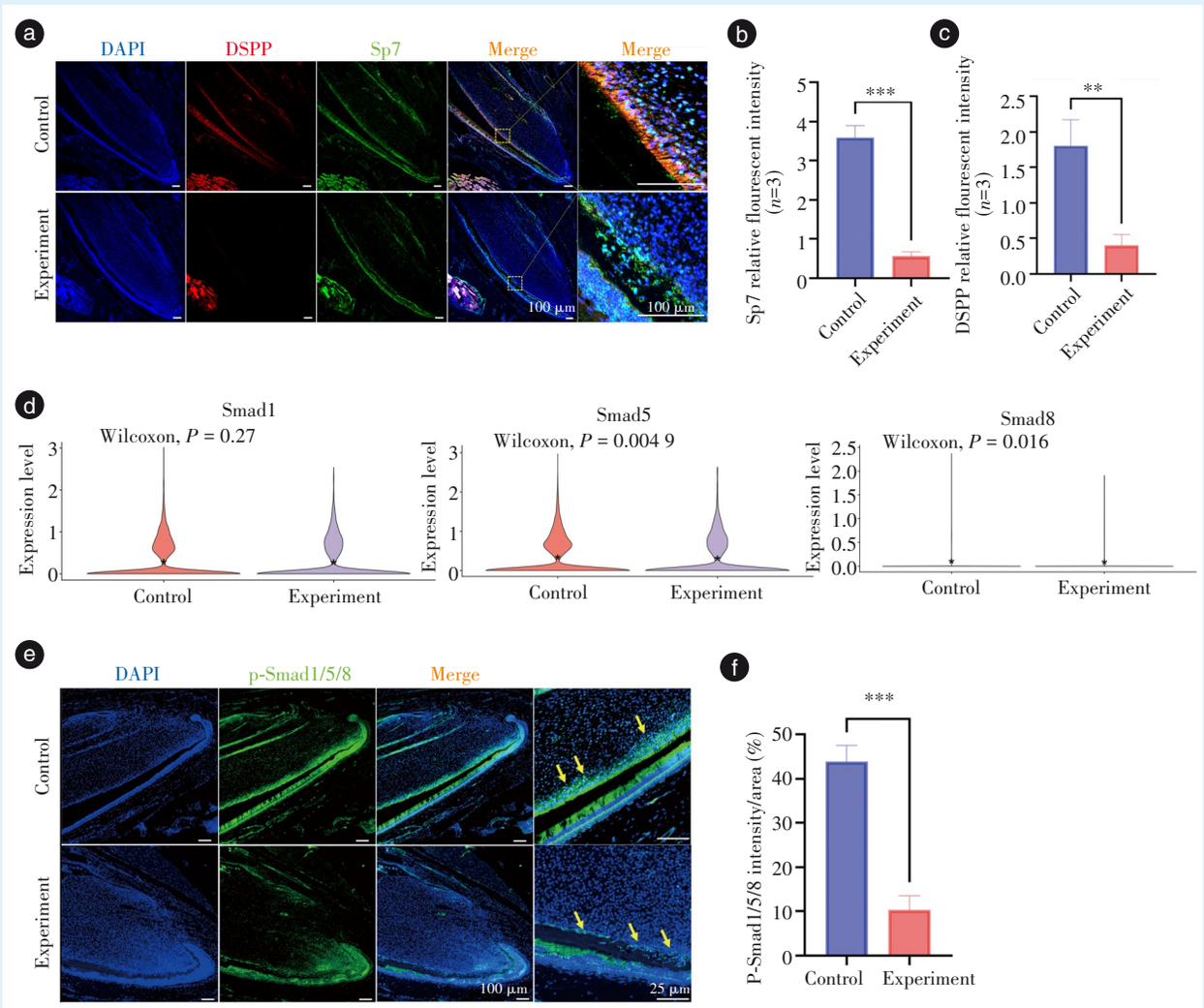
Figure 7　ACVR1 deletion disrupts the ACVR1-Sp7 axis

图7　ACVR1 缺失破坏 ACVR1-Sp7 调控轴



This study used the Cre Loxp system to construct an ACVR1 conditional knockout mouse model. Experiment group: Osterix Cre; $ACVR1^{fx/-}$ mice, con trol group: Osterix Cre; $ACVR1^{fx/+}$ mice. All mouse strains are C57BL/6J, sourced from Yuji Mishina Laboratory at the School of Dentistry, University of Michigan, USA. a: immunofluorescence staining further confirmed markedly reduced expression of Sp7 and DSPP in the incisor dental pulp of the experimental group. The yellow arrow points to the odontoblast layer and the yellow box represents the enlarged portion of the field of view; b-c: quantitative fluorescence analysis of Sp7 and DSPP, n = 3 mice per group; d: changes in the expression levels of Smad1, Smad5, and Smad8 at the single-cell level, n = 4 mice per group; e-f: changes in p-Smad1/5/8 expression levels observed *via* immunofluo rescence staining, n = 3 mice per group. ** $P < 0.01$; *** $P < 0.001$

Figure 8　Decreased expression of Sp7 transcription factor and DSPP protein in the mesenchyme of ACVR1 deficient mice

图8　ACVR1缺失小鼠间充质中Sp7转录因子及DSPP蛋白表达下降

不可或缺的调控作用，其关键机制之一在于直接激活下游牙本质特异性基因的表达，其中尤为重要的是DSPP[14]——牙本质中最主要的非胶原蛋白，对牙本质的矿化与结构构建具有决定性影响[15]。

小鼠出生后其切牙能不断生长，这一特性部分得益于间充质细胞持续向成牙本质细胞转变这一生理过程，因此，小鼠切牙是探究成牙本质细胞与其他间充质细胞异质性的理想体内模型[16-17]。随着单细胞组学在牙发育研究中的普遍应用，单细胞分辨率下获得的信息有利于更好地探究基因在间充质发育过程中发挥的作用[18-19]。蛋白ACVR1是转化生长因子-β（transforming growth factor-β，TGF-β）超家族中的一种Ⅰ型受体，涉及神经性疼痛、血管生成、组织修复等多种生物过程[20-22]。本研究通过整合单细胞转录组分析与组织学验证，系统揭示了ACVR1在牙本质形成，特别是在成牙本质细胞分化过程中的关键调控作用。

通过对公共单细胞数据集(GSE146123)的生物信息学挖掘,预测并证实了ACVR1与一系列成牙相关基因,尤其是与成牙本质细胞分化的核心转录因子Sp7,存在正相关性。这一发现提示ACVR1可能参与了调控成牙本质细胞分化的基因网络。进一步的通路富集分析表明,ACVR1与多个和细胞分化、发育相关的信号通路密切关联,为其在牙本质形成中的调控作用提供了潜在的通路背景[23-24]。

随后,在本实验构建的实验模型中,ACVR1在牙髓间充质细胞中的条件性敲除,导致了牙髓细胞群体结构的显著改变。作为终末分化的成牙本质细胞的直接前体,前成牙本质细胞已继承了调控牙本质生成的关键基因表达特征[25-26]。在本研究中最为突出的表现是,前成牙本质细胞亚群的比例异常增加,但与此同时该群体中标志成牙本质细胞成熟与极化的关键基因[14, 27-28],如Sp7、Ras同源基因家族成员A(Ras homolog family member A,RhoA)、Cdc42等表达却普遍下调。这一看似矛盾的现象可能揭示了一个重要的生物学过程:ACVR1的功能缺失虽然推动了间充质细胞向成牙本质细胞谱系的早期命运决定或扩增(导致前成牙本质细胞群扩大),但严重损害了该谱系细胞后续的终末分化和功能成熟。ACVR1可能是协调成牙本质细胞"数量扩增"与"质量成熟"平衡的一个关键开关。本实验结果进一步支持上述假设。在实验组中,不仅前成牙本质细胞内的分化基因下调,整个间充质细胞谱系中Sp7的表达均呈现系统性降低。此外,其他多个与ACVR1表达正相关且已知参与颅颌面发育与细胞分化的转录因子[29-31],如Msx1、Sox9、Distal-less同源框5(Distal-less homeobox 5,DLX5)也同步下调。这表明ACVR1的功能缺失可能通过影响核心的转录调控网络,从而广泛地抑制了成牙本质细胞的成熟程序。DSPP作为由成牙本质细胞特异性合成与分泌的前体蛋白[32],经酶切加工后生成牙本质磷蛋白(dentin phosphoprotein,DPP)与DSP[33-34]。其表达丰度与时空模式,直接决定了细胞外基质中胶原纤维的矿化效率、晶体排列的有序性以及最终牙本质的微观结构与力学性能[35],因此DSPP是评估牙本质形成质量的关键分子指标。最终通过免疫荧光染色在组织层面证实:实验组牙髓中Sp7及其下游靶基因DSPP的蛋白表达水平显著降低,直观地反映了牙本质形成功能的受损。

综上所述,本研究确立了ACVR1是成牙本质细胞正常分化和功能成熟所必需的调控因子。其机制可能涉及通过调控以Sp7为核心的分化转录网络[36-37],以及与Msx、Rhoa等因子的协同作用,从而确保成牙本质细胞在适当的时间和位置完成极化、分泌基质等关键步骤[38-39]。本研究在发现ACVR1缺失导致Sp7下调及前成牙本质细胞比例增加的基础上,揭示了ACVR1与Msx1、Msx2的表达相关性。鉴于牙发育中BMP、Wnt及FGF等通路存在广泛联系[40],且Msx家族是Wnt通路的潜在交互节点[41],推测ACVR1对成牙分化的调控可能不局限于BMP/Smad轴。据此提出一个假设性模型:ACVR1缺失可能通过削弱BMP信号,改变下游Smad1/5/8的磷酸化活性,导致Msx1、Msx2转录因子的表达下降;鉴于Msx参与Wnt/β-catenin信号转导,该变化可能进一步扰乱Wnt通路稳态,最终导致Sp7表达失调及成牙本质细胞分化受阻。后续需重点探究ACVR1缺失背景下,牙乳头间充质或成牙本质细胞中Wnt/β-catenin活性的变化,以明确该交互网络在牙本质发生中的调控机制。

【Author contributions】 Song SY designed the study, performed the experiments and wrote the article. Liu CW, Shi C, Liu QL, Sun HC designed the study, performed the experiments and reviewed the article. All authors read and approved the final manuscript as submitted.

【Ethics Statement】 All animal procedures were carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org) and adhered to the guidelines for the care and use of laboratory animals issued by the China Animal Research Council. All animal experiments were approved by the appropriate committees of Approved by the Experimental Animal Ethics Committee of Jilin University School of Basic Medicine (Approval ID: 2026(No.4)).

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【Data availability statement】 Part of the data used in this study comes from the Gene Expression Omnibus database (https://www.ncbi.nlm. NIH. gov/go/), with dataset ID: GSE146123. The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author upon reasonable re-

quest.

【**Generative AI statement**】 The authors declared that generative AI was not used in the creation of this manuscript.

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