



小鼠次级卵母细胞和第一极体的高分辨率差异蛋白质组学分析*

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【摘要】目的 通过高分辨率单细胞蛋白质组学技术对小鼠次级卵母细胞与第一极体的蛋白质组差异进行分析, 筛选出调控胚胎发育的关键特征蛋白, 为优化卵母细胞体外成熟体系提供分子依据。**方法** 采用高分辨率单细胞质谱仪(timsTOF HT)对5组配对样本(次级卵母细胞与第一极体)进行蛋白质组检测, 通过定量分析结合生物信息学方法完成差异蛋白筛选及功能富集分析。**结果** 该技术实现单样本3 000余种蛋白质的检测灵敏度, 并成功鉴定出277种次级卵母细胞特异性表达蛋白。基因集富集分析(Gene Set Enrichment Analysis, GSEA)显示, 这些特异性蛋白在线粒体能量代谢相关基因调控及DNA损伤修复通路呈现显著富集趋势。与人鼠同源蛋白GSEA对比发现, mTORC1通路中Hsp90b1、Hspa5等热休克蛋白在次级卵母细胞中呈现高表达($P < 0.05$)。同时, Calr、Aldoa等调控卵丘复合体发育的关键因子也显著上调。**结论** 本研究通过timsTOF HT技术对卵母细胞不对称分裂过程中策略性保留胚胎发育所必需的蛋白进行了检测与鉴定, 该技术具有高灵敏度等优点。对蛋白的分析结果表明, mTORC1通路蛋白的分布提示其在胚胎代谢调控中的关键作用, 其中热休克蛋白通过促进蛋白质折叠及维持内质网稳态, 保障卵母细胞的成熟与胚胎发育潜能。

【关键词】 单卵母细胞 蛋白质组学 极体 次级卵母细胞

High-resolution Differential Proteomic Analysis of Mouse Secondary Oocytes and First Polar Bodies

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[Abstract] Objective To analyze the proteomic differences between mouse secondary oocytes, also known as metaphase II oocytes (M II), and the first polar bodies (PB1) using high-resolution single-cell proteomics, to identify key proteins regulating embryonic development, and to provide a molecular basis for optimizing *in vitro* oocyte maturation systems. **Methods** Paired samples of M II ($n = 5$) and PB1 ($n = 5$) were analyzed using high-resolution single-cell mass spectrometry (timsTOF HT). Quantitative proteomics and bioinformatics approaches were employed to conduct differential protein screening and functional enrichment. **Results** Using the timsTOF HT platform, we achieved the detection of over 3000 proteins per single cell and identified 277 proteins specifically enriched in M II. Gene Set Enrichment Analysis (GSEA) revealed that these M II-specific proteins were significantly enriched in gene regulation and DNA damage repair pathways associated with mitochondrial energy metabolism. Cross-species GSEA comparison between human and mouse homologs demonstrated elevated expression of heat shock proteins, including Hsp90b1, Hspa5, etc., in the mTORC1 pathway in M II ($P < 0.05$). In addition, key factors regulating cumulus complex development, such as Calr, Aldoa, etc., were significantly upregulated. **Conclusion** M II strategically retains proteins essential for embryonic development through asymmetric division. The timsTOF HT platform demonstrated superior sensitivity in analyzing and identifying these proteins. According to the protein analysis results, the distribution of mTORC1 pathway proteins indicates that they play a key role in embryonic metabolism regulation. In particular, heat shock proteins facilitate protein folding and maintain endoplasmic reticulum homeostasis, thereby ensuring oocyte maturation and the embryonic developmental potential.

[Key words] Single-cell oocyte Proteomics Polar body Secondary oocyte

卵母细胞的产生、发育、成熟直至受精这一过程的调控机理始终是生殖领域研究关注的焦点^[1]。这一过程不仅涉及减数分裂的时空协调性, 更依赖于胞质内蛋白质网络的动态重构^[2]。传统研究多聚焦于基因层面的单

通道解析模式, 虽揭示了部分关键调控因子^[3-4], 但无法捕捉蛋白质特有的翻译后修饰、亚细胞定位及代谢周转规律——这些特性直接决定了卵母细胞极性建立、减数分裂纺锤体组装等关键生物学事件^[5-6]。尤其在第一极体排出阶段, 次级卵母细胞通过不对称分裂保留95%以上的胞质组分, 这种独特的胞质分配模式提示, 极体可能不仅是染色体分离的副产物, 其蛋白质组特征或蕴含调控卵

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子发育能力的潜在信息^[7-8]。由于传统蛋白质组学检测技术存在灵敏度不足的局限性,目前针对第一极体蛋白质组的研究数据仍较为匮乏,这阻碍了卵母细胞成熟质量评估体系的系统构建。

近年来,单细胞分析技术的突破为破解这一难题提供了全新视角。尽管单细胞转录组学已广泛应用于卵母细胞发育的研究^[9],但mRNA丰度与功能蛋白表达间存在显著的解耦现象^[10]。例如,调控减数分裂恢复的CDK1激酶活性受磷酸化修饰调控,其功能状态无法通过转录组数据推断^[11]。单细胞蛋白质组学技术的兴起,特别是基于质谱的超高灵敏度检测平台,使得在单个卵母细胞(直径约80 μm)甚至极体(直径约5~10 μm)中实现数千种蛋白质的定量分析成为可能^[12]。这一技术突破不仅克服了卵母细胞样本稀缺性的瓶颈,更揭示了传统批量检测所掩盖的细胞异质性,为解析卵母细胞成熟过程中的蛋白质时空动态提供了敏感且高效的分辨率^[13]。

因此,本研究利用高分辨率单细胞级蛋白组学技术检测小鼠次级卵母细胞及其排出的第一极体中的蛋白质,并筛选出了次级卵母细胞独有的蛋白类群。同时结合人鼠同源蛋白进行综合分析,这些发现不仅完善了卵母细胞不对称分裂的分子图谱,还为临床胚胎质量评估提供了可转化的蛋白标志物组合。

1 材料与方法

1.1 样本制备

本研究经复旦大学生命科学学院实验动物伦理委员会批准(审批号:2022JS071)。选取5只6周龄性成熟C57雌鼠[体质量(18 $\pm$ 1) g,北京维通利华],通过腹腔注射5 IU孕马促性腺激素(PMSG, 0.1 mL/只)。48 h后追加注射5 IU人绒毛膜促性腺激素(HCG, 0.1 mL/只)。注射HCG 13 h后采用颈椎脱臼法处死小鼠,完整取出双侧卵巢。

在体视显微镜下使用27G吸卵针穿刺卵泡,收集形态均一的GV期卵母细胞。用含0.1 mg/mL透明质酸酶的M2培养基处理1 min,待颗粒细胞完全解离后,将脱颗粒卵母细胞转移至培养液(TCM-199培养基,含10%胎牛血清、2 mmol/L谷氨酰胺、0.23 mmol/L丙酮酸钠、10 IU/mL PMSG、50 IU/mL青霉素-链霉素)。接着采用微滴培养体系(80 μL 培养液/滴,表面覆盖矿物油),将卵母细胞置于37.5 $^{\circ}\text{C}$ 、体积分数为5% CO_2 、饱和湿度条件下培养。

培养13 h后,在倒置相差显微镜下筛选具有完整卵周隙且排出第一极体的M II期卵母细胞。使用0.3%稀盐酸溶液消化透明带后,通过显微操作系统(Eppendorf TransferMan[®] 4r)分别将次级卵母细胞及其对应第一极

体转移至预冷的0.2 mL EP管中。

1.2 单细胞样本处理

配制单细胞裂解液(scMix): 87 μL 超纯水、10 μL 1 mol/L HEPES(pH7.4)、2 μL 十二烷基麦芽糖苷(DDM)及1 μL 胰蛋白酶(1 $\mu\text{g}/\mu\text{L}$)。用预冷PBS清洗细胞三次后,每管加入1.5 μL scMix裂解液,立即密封管口并用封口膜加固,液氮速冻后转移至-80 $^{\circ}\text{C}$ 保存。

1.3 单细胞质谱分析

使用timsTOF HT质谱系统(Bruker Daltonics)对首批5对样本(次级卵母细胞和第一极体配对)进行单细胞级蛋白质组分析,仪器参数设置参照制造商标准操作流程。

1.4 差异数据分析

首先采用z-score法^[14]对原始蛋白表达量进行标准化处理,基于R语言(v4.2.1)的factoextra包完成主成分分析(principal components analysis, PCA),同时通过Hiplot平台(hiplot.com.cn)构建火山图及聚类热图,差异蛋白筛选采用GraphPad Prism(v9.4.1)进行独立样本双侧t检验, $P<0.05$ 为差异有统计学意义。对筛选获得的差异表达蛋白进一步通过Metascape平台进行功能注释,采用超几何检验($P<0.01$)结合富集因子>1.5的标准,进行GO功能富集分析。基因集富集分析(Gene Set Enrichment Analysis, GSEA)采用Broad Institute开发软件(v4.3.2),以KEGG数据库为参考解析生物学通路激活状态。

2 结果

2.1 次级卵母细胞及第一极体蛋白富集差异明显

使用R语言代码将两组蛋白进行PCA主成分分析(图1),发现第一极体的组内差异较小、相似性高,次级卵母细胞组内相似性稍差,但大多靠近置信区域。次级卵

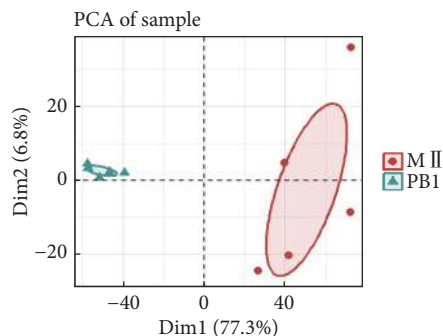


图1 次级卵母细胞及第一极体的主成分分析

Fig 1 Principal component analysis(PCA) of secondary oocytes (M II) and first polar bodies (PB1)

M II: secondary oocytes, also known as metaphase II oocytes; PB1: first polar body.

母细胞组与第一极体组的组间差异明显。

热图分析两组蛋白(图2),发现5组次级卵母细胞及第一极体的组内聚类好,且第一极体中的蛋白含量远少于次级卵母细胞组,因此推测卵母细胞第一次减数分裂中,将胞质中的蛋白不均等分裂,留下的特异性蛋白将发挥重要作用。接下来本研究筛选出次级卵母细胞组中特有的蛋白,并进行生物信息学分析。

2.2 次级卵母细胞特有蛋白的生物信息学分析

两组蛋白数据经过组蛋白归一化处理后,共分析得到3958个蛋白,其中次级卵母细胞特有蛋白有277种。根据平均含量进行排序后,含量前10的蛋白分别是: Pole2、Tep1、Pi4k2b、Tk2、Abi2、Pdcd2、Bcl7c、Btbd16、Noct、Klhl8,其主要功能有代谢、定位、应激、免疫、稳态、细胞过程以及生物过程的正负调控等。接着,本研究将次级卵母细胞特有蛋白进行GSEA,发现30多条相关蛋白通路(图3),其中富集最多的通路有线粒体基因表达(GO: 0140053)、mRNA的代谢过程(GO: 1116071)、靶向卵液蛋白(GO: 0006623)、RNA聚合酶 II 启动子的转录起始点(GO: 0006367)、DNA双链断裂修复(R-

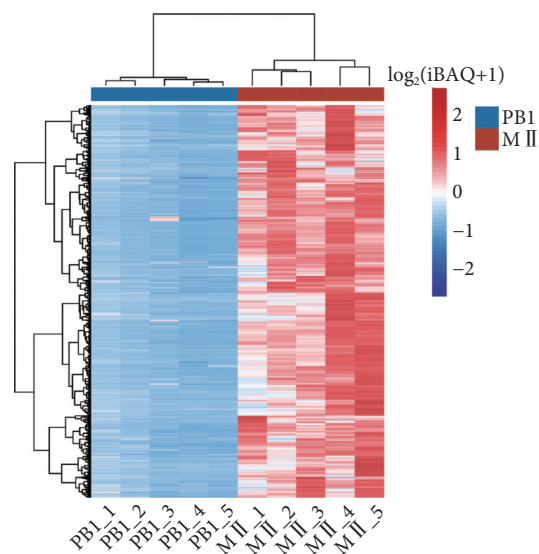


图2 次级卵母细胞及第一极体的热图分析

Fig 2 Heatmap analysis of secondary oocytes (M II) and first polar bodies (PB1)

MMU-5693532)以及端粒的延伸(R-MMU-180786)等。次级卵母细胞将线粒体、核酸表达及代谢、DNA损伤修复相关蛋白保留,这将为后续的细胞发育、受精、早期胚胎细胞分裂及谱系分化提供物质储备。

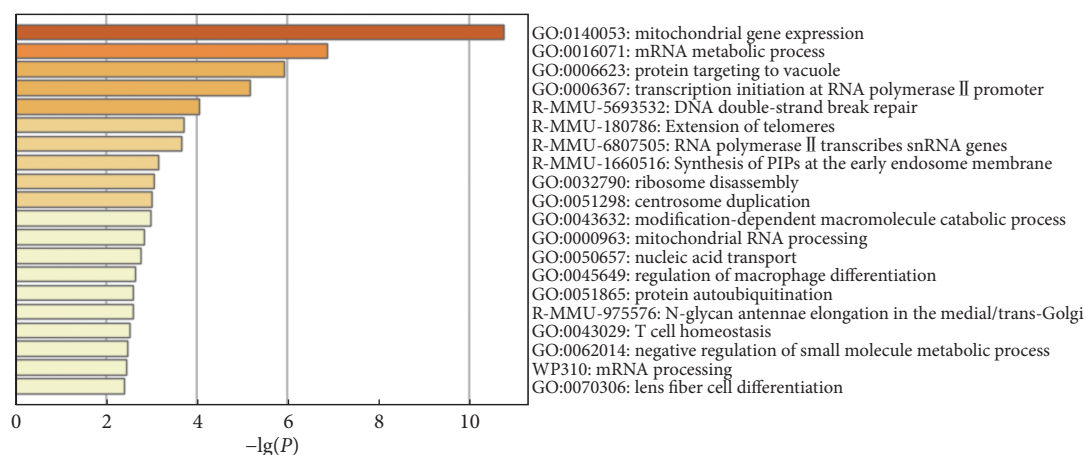


图3 次级卵母细胞特有蛋白的GO富集分析

Fig 3 GO enrichment analysis of secondary oocyte-specific proteins

2.3 火山图分析次级卵母细胞及第一极体中的差异蛋白

除了次级卵母细胞特有的蛋白,本研究还对次级卵母细胞及第一极体均表达的蛋白进行DESeq2差异分析。将次级卵母细胞与第一极体对比后,发现差异有统计学意义的蛋白共198种($P < 0.05$, $|\log_2FC| > 1.00$),上调蛋白有68种,下调蛋白有130种。绘制了火山图(图4)后,发现次级卵母细胞和第一极体的蛋白组组内相关性好,组间差异明显。因人鼠卵母细胞蛋白组的差异,接下来本研究将小鼠次级卵母细胞对比第一极体的差异蛋白与人源蛋白进行分析比较,发掘其富集的蛋白通路。

2.4 GSEA分析次级卵母细胞差异蛋白功能

因人卵母细胞获取困难且涉及伦理问题,所以本研究主要针对小鼠卵母细胞,为探究与人卵母细胞的相关性,后续本研究基于人鼠同源蛋白数据库,对所有数据进行GSEA和热图聚类分析发现: mTORC1信号通路蛋白类群在次级卵母细胞(M II)内相对于第一极体(PB1)有显著富集,其中热休克蛋白家族(Hspa5、Hsp90b1、Hspa9、Hspe1等)、Clar蛋白、Aldoa蛋白显著富集(图5A、5B),其中Hspa5蛋白与Hsp90b1蛋白表达量较高(图5C),这也同样说明了次级卵母细胞将相关的信号通路蛋白倾向性地

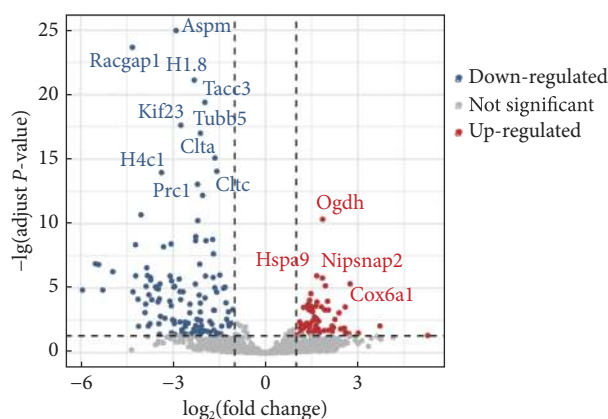


图 4 小鼠次级卵母细胞与第一极体的火山图分析

Fig 4 Volcano plot analysis of mouse secondary oocytes and the first polar bodies

留存于细胞胞质中,从而提高蛋白代谢、应对内质网的应激以及维持细胞内环境稳态等,为后续卵母细胞的成熟及受精做好准备。

3 讨论

本研究通过高分辨率单细胞蛋白质组学技术(timsTOF HT质谱仪)实现了检测灵敏度的突破,单个样本定量蛋

白数达3000余种。相较于PEDDINTI等^[15]在牛GV期卵母细胞中鉴定的1950种蛋白,MA等^[16]在小鼠M II期发现的380种银染蛋白,以及CAO等^[17]采用SDS-PAGE+LC-MS/MS技术建立的1405个小鼠GV期蛋白表达谱,该方法能获得更为丰富和全面的数据,系统揭示了小鼠次级卵母细胞与第一极体的蛋白质分布特点,未来将对卵母细胞的蛋白谱建立提供更加精确的数据参考。值得注意的是,本研究将检测对象扩展至第一极体,为解析卵母细胞不对称分裂的分子机制提供了全新视角。

研究结果发现,次级卵母细胞与其排出的第一极体蛋白差异明显,并且次级卵母细胞特异性保留的277种蛋白主要富集于线粒体基因表达、核酸代谢及损伤修复等关键生物学过程,这与既往发现的TRIP13^[18]、REC114^[3]及ZP1^[19]等减数分裂调控基因形成功能互补。通过GSEA分析发现的mTORC1信号通路在次级卵母细胞中显著富集,其中Hspa5与Hsp90b1表达较高,为解释卵母细胞成熟过程中能量代谢与发育调控的偶联机制提供了蛋白水平证据。mTORC1信号通路是人体重要的信号通路,通过促进葡萄糖、蛋白质及脂质合成与代谢等方式来促进细胞生长与增殖^[20-21],同时有研究表明,它对卵泡发生及卵

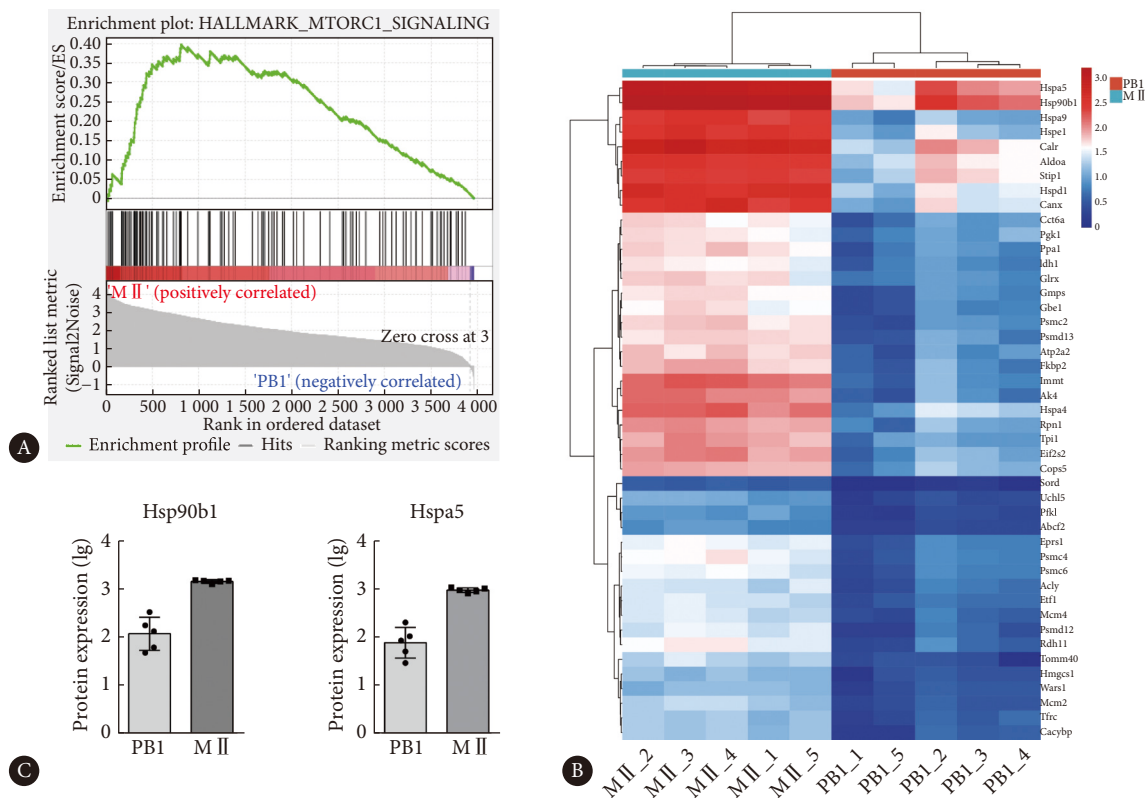


图 5 次级卵母细胞和第一极体mTORC1信号通路差异分析

Fig 5 Differential analysis of mTORC1 signaling pathway in secondary oocytes (M II) and first polar bodies (PB1)

A, GSEA of the mTORC1 signaling pathway in M II and PB1; B, heatmap plot of significantly different proteins clustered in the mTORC1 signaling pathway; C, protein expression of Hsp90b1 and Hspa5 between mouse M II and PB1 ($n = 5$).

母细胞成熟等卵巢功能均发挥着重要的作用^[22]。Hsp90b1(也称为Grp94、Erp99)是一种重要的内质网伴侣蛋白,与Hspa5(也称为Grp78、BIP)协同促进细胞内质网中蛋白质折叠^[23-24]。AUDOUARD等^[25]研究证实,Hsp90b1的蛋白活性对维持胚胎正常发育周期具有关键调控作用,其通过防止G₂/M期进程延迟和抑制减数分裂异常等机制保障细胞分裂质量。实验数据显示,Hsp90b1缺失会直接破坏微管纺锤体的结构完整性,并引发纺锤体周围肌动蛋白-内质网网络的空间定位紊乱。研究证明Hsp90b1是小鼠胚胎成功完成首次有丝分裂的必要分子基础^[25-26]。Hspa5是内质网应激响应的关键调控因子,通过帮助折叠和组装新合成的蛋白质,确保内质网环境的稳定,从而减少因应激导致的细胞损伤^[27-28]。卵母细胞在成熟过程中需要大量的蛋白质,包括卵母细胞特异性蛋白和即将植入胚胎所需的蛋白。Hsp90b1和Hspa5作为分子伴侣,帮助这些蛋白质正确折叠和组装,确保其功能的正确发挥^[29]。因此,热休克蛋白家族通过多种途径确保卵母细胞在成熟过程中能够维持其完整性和功能,为随后的受精和胚胎发育打下坚实基础^[30-31]。除了HSP家族蛋白外,Calr、Aldoa、Stip1、Canx蛋白在次级卵母细胞中显著富集,其中,Calr在卵母细胞中促进TGF- β 家族蛋白GDF9和BMP15的成熟,并且对卵母细胞-卵丘复合体(COC)的发育和女性生育能力发挥重要作用^[32]。目前研究大多聚焦于小鼠卵母细胞GV期至M II期的蛋白质组动态变化,例如,LI等^[13]对小鼠GV期、GV破裂(GVBD)期和M II期阶段的单个细胞进行单细胞蛋白质组分析,单个卵母细胞中鉴别出1500种蛋白。但对于其中排出的第一极体蛋白报道尚少,因此,本研究通过对比次级卵母细胞与第一极体的蛋白组成差异,揭示了母源蛋白选择性保留的精细调控模式,这对完善卵母细胞体外成熟培养体系具有重要参考价值。

在方法学层面,本研究验证了单细胞级质谱技术在微量样本分析中的优势。相较于传统银染技术58.8%的可分析率^[21],timsTOF HT的高分辨率特性有效克服了微量样本检测的技术瓶颈。但需指出,本研究样本量(5组)仍存在统计学局限性,且筛选出的特征蛋白尚未进行功能验证,这些均为后续研究指明了方向。未来通过扩大样本规模并结合基因编辑技术,可进一步验证母源蛋白选择性保留的生物学意义。另外,后续可以将蛋白组数据整合单细胞转录组与翻译组数据,构建卵母细胞成熟的多组学调控网络。

综上所述,本研究基于高分辨率单细胞蛋白质组学技术为卵母细胞成熟机制提供新的思路,为卵母细胞第

一次减数分裂缺陷的机制研究提供蛋白数据支持,未来可对次级卵母细胞及第一极体的生理蛋白图谱的构建、临床标志物的开发提供参考。

* * *

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