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卵巢雌激素缺乏经调节海马线粒体功能与胞外基质重塑参与阿尔茨海默病发病与进程

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[摘要] **目的** 绝经后女性阿尔茨海默病(Alzheimer's disease,AD)发病率显著升高,绝经导致的卵巢雌激素缺乏被认为是AD致病风险的主要原因之一,但是卵巢雌激素调节与学习记忆密切相关的海马的机制尚无详细的报道。本研究旨在通过整合海马转录组学与蛋白质组学分析,探讨卵巢切除(ovariectomy,OVX)所致的雌激素缺乏促进AD发生的关键分子和通路。**方法** 构建模拟绝经的OVX小鼠模型,提取海马mRNA和蛋白分别进行转录组学测序(n=3)和蛋白质组学测序(n=3),差异基因/蛋白的阈值设为差异倍数变化>1.5且P<0.05。然后将双组学共趋势分子进行GO、KEGG富集及聚类等生物信息学分析筛选关键通路;最后将共趋势分子与人类AD数据库(AlzDate)海马差异基因数据集进行表达趋势比对,将变化趋势一致的基因利用string、GeneCards/Uniports等在线资源进行蛋白互作与网络分析,筛选关键分子。**结果** 转录组学鉴定出139个差异表达基因,蛋白质组学鉴定出248个差异表达蛋白,其中18个分子在两组学中表达趋势一致。功能富集分析显示,这些共趋势分子主要参与轴突生成、细胞骨架动态调节及长链脂肪酸转运。与人类AD数据库比对后,发现18个共趋势分子中11个基因与人类海马AD数据库筛中的差异基因表达一致,互作网络鉴定出*Aldoc*、*Aldh6a1*、*Etnppl*和*Itih3*是关键的基因,这些基因分别参与糖酵解、线粒体能量代谢、氧化应激及透明质酸代谢介导的细胞外基质重塑;另有3个差异分子(Prxl2a、Set、Plin4)在AD患者海马数据库中无记录。**结论** 绝经后卵巢雌激素缺乏可能通过下调海马*Aldoc*、*Etnppl*和*Aldh6a1* 3个关键基因影响线粒体能量代谢、下调海马*Itih3*基因表达介导细胞外基质稳定性,上述各种效应协同抑制海马轴突再生能力、破坏细胞骨架稳定性、阻碍长链脂肪酸转运,最终驱动AD的发生和病理进程。

[关键词] 绝经;卵巢切除;海马;多组学分析;阿尔茨海默病

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Ovarian estrogen deficiency participates in the pathogenesis of Alzheimer's disease by regulating hippocampal mitochondrial function and extracellular matrix remodeling

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[**Abstract**] Objective The incidence of Alzheimer's disease (AD) in postmenopausal women is significantly increased with ageing. Menopause induced ovarian estrogen deficiency has been regarded as one of the causative risk factor of AD, but currently the detail mechanisms underlying estrogen regulation on the hippocampus remain unclear. This study aimed to uncover the potential pathways and key molecules through which estrogen deficiency induced by ovariectomy promotes AD pathogenesis by integrating transcriptomic and proteomic analyses. Methods The ovariectomy (OVX) mouse model was established to simulate postmenopausal estrogen decline. Transcriptomic (n=3) and proteomic (n=3) differences in the hippocampal tissues of OVX mice were analyzed using RNA sequencing and protein mass spectrometry. Co-trend molecules identified from both omics datasets were subjected to GO/KEGG enrichment and cluster analyses. These molecules were further compared with the human AD hippocampal database (AlzDate) for expression trend consistency; and online resources such as String, GeneCards/Uniports were employed to analyze protein interactions and networks of the co-trend genes to identify key molecules. Results A total of 139 differentially expressed genes (DEG) and 248 differentially expressed proteins (DEP) were identified, with 18 molecules showing consistent expression trends in both omics. Functional enrichment analysis revealed that these molecules were primarily involved in axonogenesis, cytoskeleton dynamics regulation, and long-chain fatty acid transport. After comparison with the human AD database, 11 of the 18 co-trend molecules exhibited expression inhibition consistent with AD-associated genes in the human hippocampal databases. Interaction network analysis identified *Aldoc* (involved in glycolysis), *Aldh6a1* (involved in ROS scavenging), *Etnppl* (involved in mitochondrial energy metabolism), and *Itih3* (involved in hyaluronic acid-mediated extracellular matrix remodeling) as key genes contributing to AD pathogenesis. Additionally, 3 co-trend molecules (Prxl2a, Set, Plin4) were not detected in human AD databases. Conclusion Postmenopausal estrogen deficiency decreases the expression levels of hippocampal *Aldoc*, *Etnppl* and *Aldh6a1* then affects mitochondrial energy metabolism; and decreases levels of *Itih3* expression then disables extracellular matrix remodeling. These effects collectively impair hippocampal axonal regenerative capacity, destabilize the cytoskeleton, and hinder the transport of long-chain fatty acids, ultimately driving the progression of AD pathology.

[**Key words**] menopause; ovarian removal; hippocampus; multiomics analysis; Alzheimer's disease

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众所周知,随着人口老龄化的来临,绝经后女性认知功能的下降给全球的医疗系统带来了重大挑战[1]。阿尔茨海默病(Alzheimer's disease,AD)是老年神经系统退行性疾病的主要类型,其主要病理特征包括细胞外β-淀粉样蛋白(Aβ)沉积形成的老年斑、细胞内tau蛋白过度磷酸化导致神经原纤维缠结以及神经元丢失等。AD现有的治疗手段有限,以Aβ有为靶标的国外单抗药物虽已获批但只能降低早期AD的风险

且伴随严重的副作用[2];国产靶向脑-肠轴的AD新药甘露特钠胶囊(GV-971)目前已处于停工停产状态;新近开展的靶向脑膜淋巴管引流的颈深淋巴静脉吻合术(lymphovenous anastomosis,LVA)目前受到样本量小、非随机设计和方法学差异的限制,同时也没有标准化的方案和严格的临床验证[3],仅有的一项单中心前瞻性、单臂探索性研究发现术后AD患者的认知能力虽有一定改善,脑脊液生物标志物(Aβ、磷酸化Tau蛋白等)水平却没有显著下降[4],因此寻找防治老年女性AD的新靶点极为重要。

AD的发病具有明显的性别差异。国外报道女性发病率是男性的3倍[5],国内报道女性发病大约是男性的2倍[6]。基于女性AD发病主要在绝经后,绝经导致的雌激素缺乏成为AD发病的重要学说之一。海马是调节学习记忆与认知功能最重要的脑区,其结构和功能深受雌激素的影响。实验动物和临床试验均表明卵巢雌激素对依赖海马的空间学习记忆行为有重要的调节作用[7-8],但是临床的随机对照试验发现雌激素替代疗法治疗AD的安全性和有效性仍存在争议、绝经后的雌激素替代治疗不能降低AD或痴呆的风险甚至可能增加心血管疾病和乳腺癌的风险[9,10],而且目前的实验研究主要基于雌激素对海马个别特定分子表达的调控,利用高通量组学进行大规模筛查候选分子的工作相对较少。CUI等[11]在卵巢切除(ovariectomy,OVX)小鼠海马检测到29个差异蛋白,FANG等[12]在OVX大鼠海马检测到41个差异蛋白,LIU等[13]报道OVX导致小鼠海马143个差异基因,而MEY等[14]报道OVX导致APP/PS1小鼠海马出现163个差异基因,这些分子与人类AD数据库的重叠情况也未见报道。

单一的组学研究可能会受到多种因素比如研究平台、实验操作、技术噪音或样本差异等多种因素的影响,而多组学联合分析通过整合不同数据来源,尤其是针对mRNA表达与蛋白表达的非线性关系,能显著提高结果的可靠性,因此针对绝经后女性AD发病率高、致死性高、社会及家庭经济负担重、治疗手段有限等现状,本研究拟在模拟绝经的实验动物水平,用转录组学和蛋白组学的多组学联合分析的手段探讨绝经所致海马功能障碍的关键分子和信号通路,同时还与人类AD海马基因表达数据库进行差异分子的对比挖掘,为AD的防治提供新的干预靶点。

1 材料与方法

1.1 实验动物与分组

本实验经陆军军医大学动物伦理委员会的审批(AMUWEC20210129),严格按照陆军军医大学实验动物的有关伦理和规范进行。8周龄C57BL/6 SPF级雌性小鼠18只,体重20±3g。小鼠在标准实验室条件下每笼4~6只饲养,自由进食进水,饲养温度20~25 ℃,湿度50%~65%,昼夜循环(光~暗循环)各12 h。所购小鼠适应性喂养1周后,随机分为OVX组和假手术组(control, Con),每组9只。动物手术按文献报道进行[15]:小鼠经异氟烷麻醉成功后俯卧位置于加热垫上,碘伏消毒小鼠腰背部两侧皮肤,用手术刀片逐层切开皮肤及皮下组织逐层切开肌肉、腹膜,弯钳挑出子宫后,在卵巢与子宫的交界处用7-0的缝合线结扎,然后用显微剪切除卵巢(假手术组小鼠只切除与卵巢等体积的脂肪组织),还纳子宫,逐层缝合腹膜、肌层、皮下组织及皮肤,等待小鼠苏醒。术后严密观察小鼠一般情况并勤换垫料,以防感染。

上述2组小鼠处理1月后,按照伦理规范与实验要求处死小鼠后立即取出小鼠脑组织,并在冰上仔细解剖海马体,将取出的海马体迅速转移至液氮中保存。每组随机各取3只小鼠海马组织进行转录组学测序、3只小鼠海马组织进行蛋白质组学质谱分析、3只小鼠海马组织液氮预处理后放入-80 ℃冰箱保存,后期通过qRT-PCR进行转录组学差异表达基因验证。实验过程中没有发生与实验动物有关的不良事件。

1.2 试剂与仪器

总RNA提取试剂盒(BSC52M1)购自博日科技;qPCR试剂盒(11141ES60、11201ES08)购自翌圣科技公司。分析天平(FA1004)购自上海天平仪器厂;动物麻醉机(R580)购自深圳瑞沃德公司;小型高速离心机(5424)购自Eppendorf公司。

1.3 RNA提取、文库构建和测序

各组小鼠的海马组织样本(n=3)委托上海拜谱生物科技有限公司进行RNA提取及RNA-Seq测序。在提取总RNA后,将RNA加入50 μL经过DEPC处理的水进行溶解,富集带有polyA尾的mRNA。随后,通过离子打断的方式,借助二价阳离子将mRNA随机打断并合成cDNA。对双链cDNA纯化处理修复后,在3'端引入"引入碱基"并连接测序接头。筛选长度约为400~500 bp的cDNA片段进行PCR扩增,再次纯化PCR产物并构建出文库。在对文库进行质量检测后,将多样品DNA文库(multiplexed DNA libraries)进行均一化处

理并等体积混合。将混合后的文库逐步稀释并定量后,在Illumina测序仪上进行测序。

1.4 蛋白样品制备、质谱数据采集与检索

蛋白样品制备、质谱数据采集与检索委托上海拜谱生物科技有限公司完成,具体流程如下:将海马组织(n=3)置于液氮中研磨,随后分别加入适量的SDT裂解液,转移至EP管中。接着,将EP管置于沸水浴中加热3 min,再进行超声破碎处理,持续2 min。之后,将样品以16 000×g的速度离心20 min,取上清液进行定量分析。之后,对蛋白样品分别进行FASP酶解处理。酶解后的肽段脱盐处理,随后进行真空冻干。冻干后的肽段使用0.1% FA进行复溶。取适量复溶后的肽段进行色谱分离。分离后的肽段进行质谱分析。最后,通过DIA-NN软件对所有质谱数据进行合并处理,完成数据库检索以及蛋白的DIA定量分析。

1.5 生物信息学分析

转录组学使用fastp软件[16]进行筛选得到高质量数据信息,使用DESeq2软件[17]进行差异表达分析。蛋白组学筛选样本数据后,对空值进行数据填充并进行统计分析。转录组学和蛋白组学差异表达的筛选条件均设定为差异倍数的绝对值大于1.5(Fold Change(FC)>1.5或者FC<1/1.5)且P-value<0.05。双组学联合分析时,首先将蛋白组学中的差异表达蛋白转化为对应的基因名,然后利用韦恩图将转录组学差异基因、蛋白组学差异蛋白两个数据集进行重叠分析,将两个组学中共趋势的差异分子通过微生信平台(https://www.bioinformatics.com.cn/)进行GO、KEGG富集及相关的聚类分析。

1.6 双组学共趋势差异基因与人类AD数据库的比对、关键基因筛选、验证及功能定位

首先,将转录组学和蛋白组学整合分析筛选出的共趋势差异表达基因与人类AD数据库(AlzDate)中AD患者海马差异表达基因进行比对,筛选出表达趋势一致的AD相关基因,并选取一个关键基因通过qRT-PCR验证,具体过程如下:从各组小鼠海马组织中提取总mRNA,并采用Hieff® qPCR SYBR Green Master Mix(No Rox)将其逆转录为cDNA。为了对比不同基因的表达水平,使用特异性引物在qRT-PCR中对cDNA进行扩增,其中以β-actin作为内参基因。通过$2^{-\Delta\Delta Ct}$法来测定mRNA的相对表达水平。然后利用STRING数据库将筛选出的AD致病基因进行互作分析,寻找卵巢切除导致AD发生的关键基因,最后结合GeneCards/UniProt数据将关键致病基因进行亚细胞定位及功能注释。

1.7 统计学方法

本研究的统计学分析借助于软件Graphpad Pism 9.5完成,两样本比较采用t检验,P<0.05表示有统计学差异。

2 结果

2.1 转录组学测序的质控与差异表达基因

样本(n=3)完成测序后,所获得的数据通过测序平台配备的专用软件进行初步处理,转化为FASTQ格式的原始数据(Raw Data),即下机数据。随后,针对每个样本的下机数据分别开展详细的统计分析,统计结果见表1。6个样品的总体测序错误率控制在0.01%以内,Qphred值不小于20的碱基百分比占碱基总数的98.7%以上,Qphred值不小于30的碱基百分比占碱基总数的96.6%以上(见表1)。上述结果表明测序结果较好,可以进行后续分析。

表1 转录组学下机数据统计

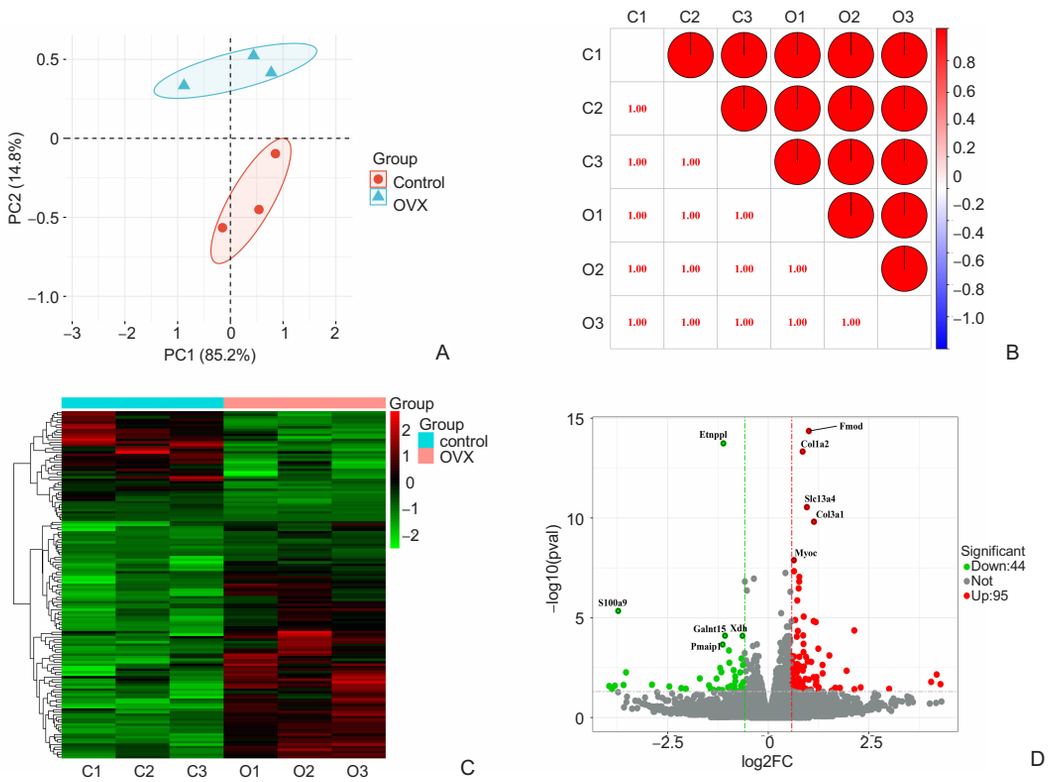
Table 1 Transcriptomic raw data statistics

Sample	Q30(bp)	N(%)	Q20(%)	Q30(%)
Con1	6658558037	0.007 154	98.84	96.74
Con2	7856719947	0.007 240	98.92	96.96
Con3	7378450262	0.007 197	98.92	96.98
OVX1	7713472056	0.007 165	98.88	96.86
OVX2	7437266127	0.007 180	98.89	96.88
OVX3	8764677649	0.007 209	98.90	96.87

为了评估组内生物学重复性和评估差异表达基因的可靠性,我们对样本进行了主成分分析(Principal Component Analysis,PCA)和Pearson相关性分析,并将皮尔逊相关系数作为生物学重复性的评估指标。从图1A中可以看出,OVX和Control组的数据点在PCA空间中呈现出不同的分布模式,表明这两组之间存在者一定的差异;同一组内样本点聚集在一起,表明组内样本之间相似度高,并且重复性好。在图1B中,两组样本的皮尔逊相关系数绝对值非常接近1,表明各处理组的重复性较好、处理因素所致的差异表达基因较为可靠。差异表达基因的聚类分析发现,OVX和Control组基因表达模式有明显的差异,且组内各样本的表达模式相似(见图1C);差异基因分析发现OVX组共有139个差异表达基因,其中显著上调基因有95个、显著下调基因44个(图1D)。

2.2 蛋白组学的质控与差异表达蛋白

为了评估蛋白组学各处理组内生物学重复性和组间的差异性,对样本(n=3)进行了PCA分析、Pearson



A: principal component analysis (PCA) of transcriptome data; B: Pearson correlation plot; C: gene expression heatmap; D: Volcano plot of differentially expressed genes, with down-regulated genes depicted in green and up-regulated genes in red. Samples C1~3: 3 control animals; O1~3: 3 experimental animals

图1 转录组学测序的质控分析、差异基因热图与火山图

Figure 1 Transcriptomic sequencing analysis: quality control, heatmap, and volcano plot.

相关性分析及差异蛋白聚类分析。从图2A中可以看出，OVX和Control组的数据点在PCA空间中呈现出不同的簇状分布，表明两组之间存在一定的差异。在图2B中，两组样本的皮尔逊相关系数绝对值非常接近1。在对差异蛋白聚类分析中发现(图2C)，OVX和Control组蛋白表达模式存在明显的差异，且组内各样本的表达模式相似。在差异蛋白的火山图(图2D)中可以发现，OVX相较于Control组共有248个差异蛋白，其中显著高表达蛋白质有96个、显著低表达蛋白质152个。

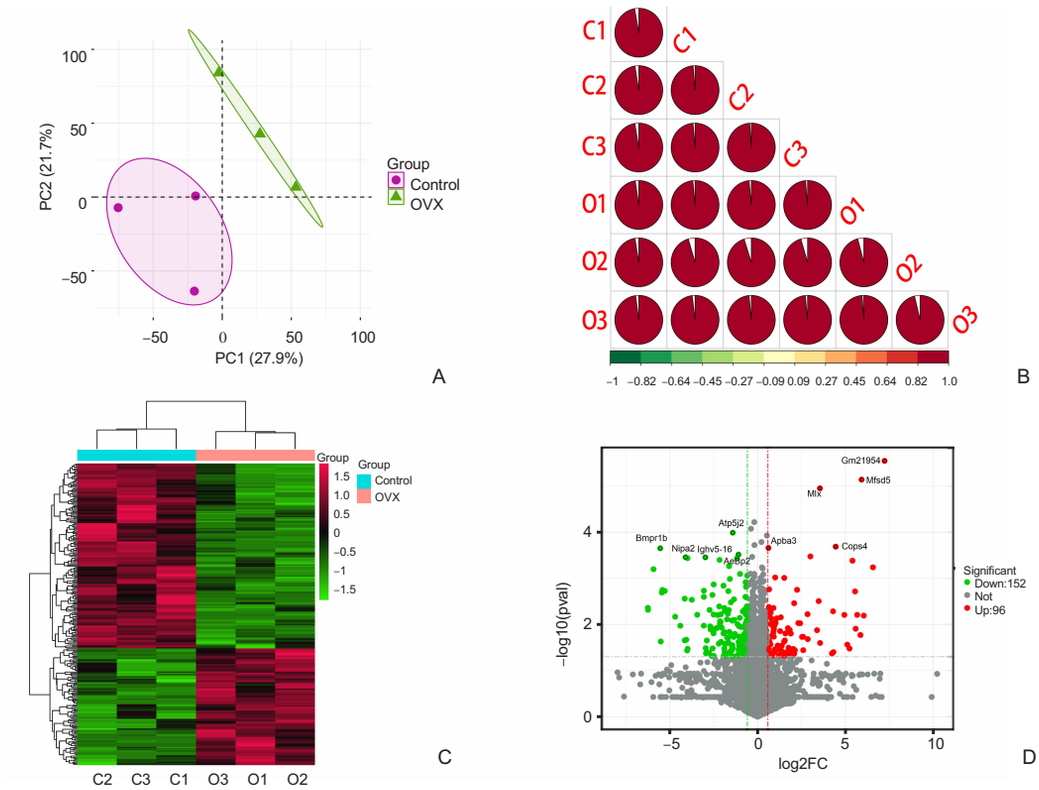
2.3 转录组学和蛋白组学的共趋势分子筛选

韦恩图显示转录组学和蛋白组学共有23个共同的差异基因/蛋白质(图3A)。其中在转录组学和蛋白组学中上下调趋势一致的有18个，即共趋势分子(图3B、C)，含上调的7个分子(Cfp、She、Sdf2l1、Sash1、Cep97、Ranbp10、Myh9)、下调的11个分子(Aldoc、Aldh6a1、Prxl2a、Fam171b、Fgfr3、Set、Cirbp、Etnppl、Itih3、Slc2a1、Plin4)。另外，上下调趋势相反的分子有5个。

2.4 共趋势分子的富集与聚类分析

我们主要对变化趋势一致的18个共趋势差异分子进行了富集分析。GO的生物学过程(Biological process, BP)富集共有200个条目，在*P*值最小的前10个条目中(图4A)，富集分数(Rich Factor, RF)最高的是神经支配相关轴突发生(RF=180.89)，其次是磷酸钠反应和骨髓细胞增殖(RF均为164.45)，p38MAPK信号通路(RF=66.99)；这200个条目进一步聚类分为11个cluster，其中神经支配相关轴突发生是最显著的cluster、包含了94个BP条目，卵子激活包含了32个条目，K63蛋白连接泛素化的调控、嘧啶核碱基代谢过程、长链脂肪酸跨膜导入和细胞对冷刺激的反应各有11个条目，按*P*值大小排名的前10个cluster见图4B。

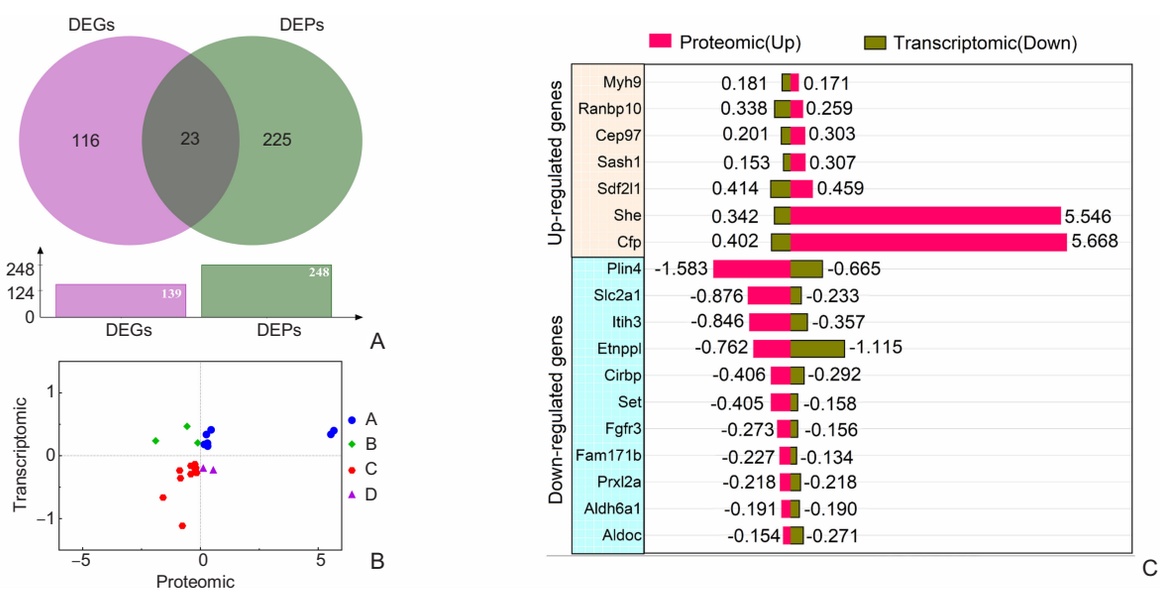
细胞组分(cell component, CC)富集总共有27个条目，*P*值最小的前10个条目(图4C)中RF最高的是雌原核(female pronucleus, RF=160.02)，其次为胞质膜泡(RF=145.47)、细胞的后缘(RF=145.47)以及内质网伴侣复合体(RF=133.35)等。这些条目进一步聚类为4个cluster，主要是肌球蛋白丝(含16个条目)，雌原核(含5个条目)，内质网分子伴侣复合体和突触后细胞质基质各自包含了2个条目(图4D)。



A: principal component analysis (PCA) plot of proteomics; B: Pearson correlation plot; C: protein expression heatmap; D: volcano plot of differentially expressed proteins, with down-regulated proteins depicted in green and up-regulated proteins in red. Samples C1~3: 3 control animals; O1~3: 3 experimental animals

图2 蛋白组学的质控分析、差异蛋白热图与火山图

Figure 2 Proteomic analysis: quality control, differential protein heatmap and volcano plot



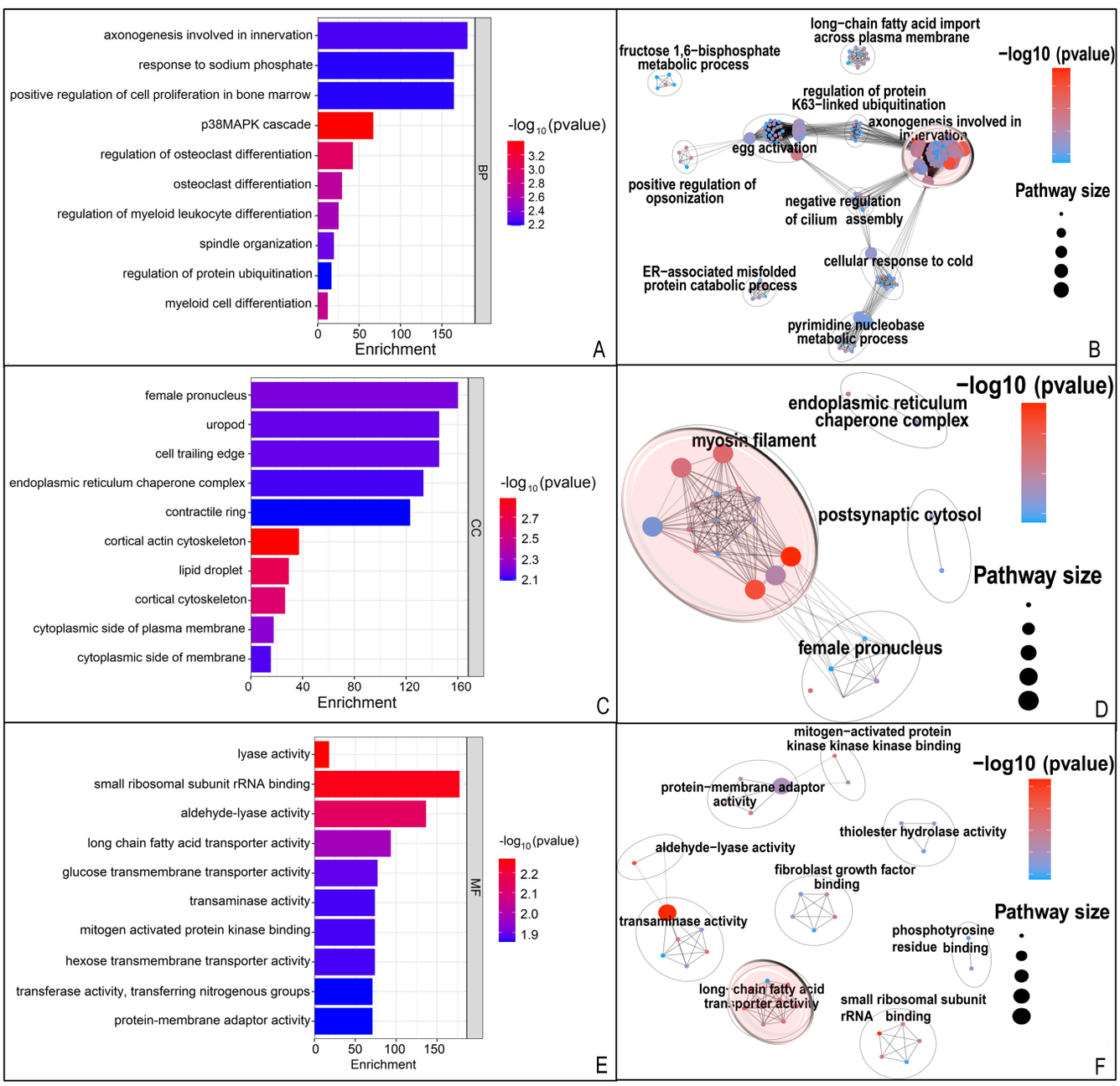
A: Venn diagram of differentially expressed genes/proteins from transcriptomics and proteomics; B: quadrant scatter plot of integrated differential molecules (Quadrant I/III: co-directional regulation; Quadrant II/IV: inverse regulation); C: bar plot of co-regulated molecule counts (numbers at bar ends indicate Log2FC of corresponding molecules)

图3 转录组学–蛋白组学整合分析筛选共趋势分子

Figure 3 Integrated transcriptomics-proteomics analysis for co-regulated molecule screening

分子功能(molecular function,MF)聚类共有41个条目,*P*值最小的前10个条目(图4E)中RF最高的是小核糖体亚基rRNA结合(RF=177.53),其次为醛裂解酶活性(RF=136.56),长链脂肪酸转运活性(RF=93.43)以及葡萄糖跨膜转运活性(RF=77.18)等。这些条目进一步聚类为9个cluster,分别为长链脂肪酸转运蛋白活性(含9个条目),转氨酶活性(含6个条目),成纤维细胞生长因子结合和小核糖体亚基rRNA结合各含5个条目等(图4F)。

KEGG富集表明这些共趋势分子富集为11个条目,*P*值最小的前10个条目主要与碳代谢、肌动蛋白细胞骨架调控、丙氨酸代谢、氧化磷酸化途径及HIF-1信号通路有关(图5A)。这些条目进一步划分为3个cluster,其中肌动蛋白细胞骨架的调控和β其丙氨酸代谢各包



A, C and E: significantly enriched terms for biological process (BP), cellular component (CC), and molecular function (MF), respectively. B, D and F: corresponding clustering results for each GO category. Color gradient: Red → Blue indicates decreasing significance (red: lowest *P*-value, blue: highest *P*-value). Red translucent circles highlight clusters with the highest term density

图4　共趋势分子GO功能富集与聚类分析结果

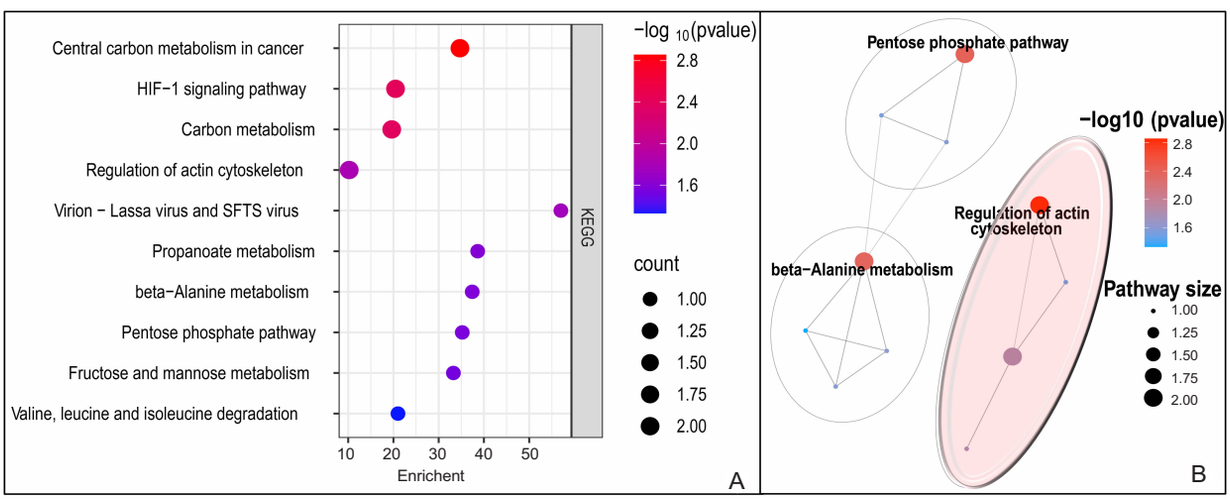
Figure 4　GO enrichment and cluster analysis of co-regulated molecules

括4个条目、磷酸戊糖途径包括3个条目,见图5B。

GO富集分析和KEGG富集分析提示,共趋势的18个分子主要与轴突生成、长链脂肪酸转运、细胞骨架动态调节有关。

2.5 共趋势分子与人类AD海马基因数据库比对及关键基因功能定位分析

为了探讨这18个分子中哪些分子参与了人类AD的发生与发展,我们首先将这18个基因与AD研究的综合知识库——AlzDate数据库中AD患者和对照人群海马差异表达标准化的数据集进行了比对,发现四个基因(*Sdf2l1*、*Ranbp10*、*Fgfr3*、*Slc2a1*)在AD患者与对照人群海马脑区差异表达趋势不一致,3个基因(Prxl2a、Set和Plin4)在AlzDate数据库无相关记录(表2、图6)。我们选择了在共趋势基因和AD海马数据库中表达均为下降的基因Etnppl,用qRT-PCR验证了其在OVX小鼠海马的表达。结果显示,与对照组相比,OVX组中



A: significantly enriched KEGG pathways; B: cluster analysis of enriched KEGG pathways; Color gradient: Red → Blue indicates decreasing significance (red: lowest *P*-value, blue: highest *P*-value). Red translucent circles highlight clusters with maximal pathway density

图5 共趋势分子KEGG富集分析结果

Figure 5 KEGG pathway enrichment analysis of co-regulated molecules

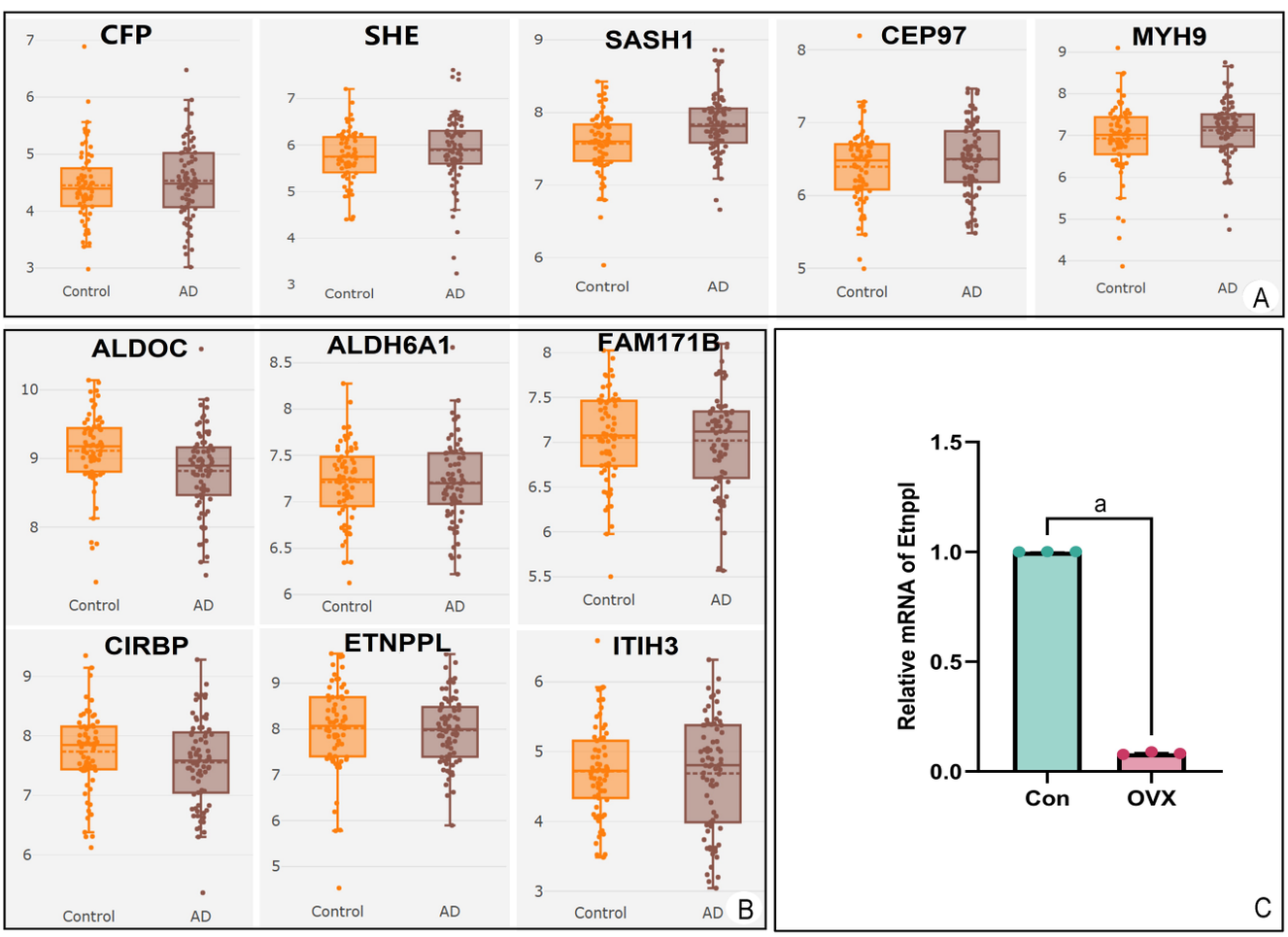
表2 共趋势分子与人类AD海马基因数据库比对

Table 2 Cross-referencing of the co-regulated molecules with human Alzheimer's disease hippocampal gene databases (AlzData)

Gene name	Proteomic		Transcriptomic		Differential expression of Hippocampus in AlzDate	
	Log2FC	P. value	Log2FC	P. value	Log2FC	P. value
Cfp	5. 668	0. 006	0. 402	0. 004	0. 156	0. 167
She	5. 546	0. 002	0. 342	0. 050	0. 103	0. 392
Sdf2l1	0. 459	0. 020	0. 414	0. 036	−0. 208	0. 195
Sash1	0. 307	0. 030	0. 153	0. 047	0. 247	1. 46E−03
Cep97	0. 303	0. 009	0. 201	0. 037	0. 07	0. 434
Ranbp10	0. 259	0. 020	0. 338	0. 000	−0. 227	0. 01
Myh9	0. 171	0. 016	0. 181	0. 023	0. 141	0. 303
Aldoc	−0. 154	0. 025	−0. 271	0. 005	−0. 258	0. 012
Aldh6a1	−0. 191	0. 000	−0. 190	0. 015	−0. 038	0. 615
Prxl2a	−0. 218	0. 022	−0. 218	0. 013	ND	ND
Fam171b	−0. 227	0. 035	−0. 134	0. 043	−0. 007	0. 939
Fgfr3	−0. 273	0. 015	−0. 156	0. 031	0. 112	0. 387
Set	−0. 405	0. 016	−0. 158	0. 026	ND	ND
Cirbp	−0. 406	0. 001	−0. 292	0. 002	−0. 165	0. 179
Etnppl	−0. 762	0. 016	−1. 115	0. 000	−0. 014	0. 922
Itih3	−0. 846	0. 005	−0. 357	0. 001	−0. 02	0. 875
Slc2a1	−0. 876	0. 033	−0. 233	0. 036	0. 124	0. 224
Plin4	−1. 583	0. 001	−0. 665	0. 001	ND	ND

Etnppl的表达明显减少(P<0.000 1),见图6C。

在转录组学和蛋白组学18个共趋势分子中,剔除与AlzDate数据库中表达趋势不一致的4个基因和无相关记录的3个基因后,我们对剩余的11个基因通过STRING数据库进行了基因/蛋白互作分析,发现Aldoc、Aldh6a1、Etnppl及Itih3这四个分子为核心分子(图7)。利用GeneCards和UniProt数据库检索(表3)表明Aldoc是大脑中主要的糖酵解酶,Etnppl催化神经酰胺、吡哆醛磷酸依赖的磷酸乙醇胺分解,Aldh6a1为三羧酸循环过程中的氧化还原酶,Itih3位于细胞外基质,参与透明质酸的代谢,进而维持ECM结构的完整性。



A: Concordantly up-regulated molecules in co-regulated set and AD hippocampal tissue (*AlzData*). B: Concordantly down-regulated molecules in co-regulated set and AD hippocampal tissue (*AlzData*). C: qRT-PCR validation of *Etnppl* expression (OVX mice show significant reduction mimicking AD hippocampal changes, n=3). a: P<0.000 1. Con: control; OVX: ovariectomized

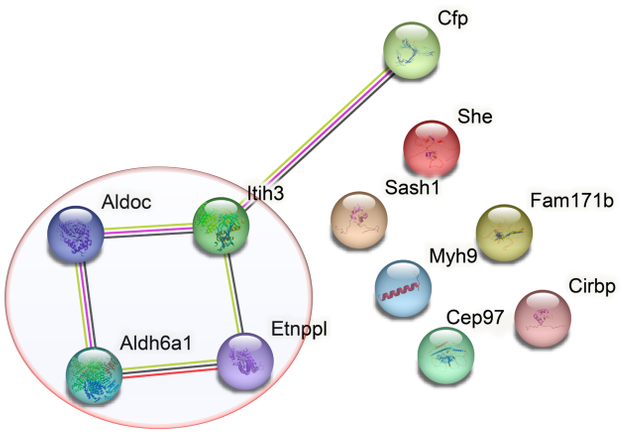
图6 共趋势分子与人类AD数据库比对结果

Figure 6 Validation of co-regulated molecules against human Alzheimer's disease databases

表3 小鼠卵巢切导致AD发生的基因功能及亚细胞定位

Table 3 Functional annotation and subcellular location of ovariectomy-induced genes contributing to pathogenesis of Alzheimer's disease in mice

Gene name	Protein name	Subcellular Location	Molecular function
Aldh6a1	Aldehyde dehydrogenase family 6 member A1	Mitochondrion/ Nucleus	Oxidoreductase
Aldoc	Fructose-bisphosphate aldolase C	Cytosol	Glycolysis
Etnppl	Ethanolamine-phosphate phospho-lyase	Mitochondrion	Ethanolamine-phosphate phospho-lyase activity
Itih3	Inter-alpha-trypsin inhibitor heavy chain H3	Collagen-containing extracellular matrix	Synthesis and degradation of hyaluronan



Aldoc, Aldh6a1, Etnppl, and Itih3 form a regulatory network with complement factor properdin (Cfp), where the former 4 proteins (red circles) constitute core regulators.

图7 小鼠卵巢切导致AD发生的蛋白互作分析

Figure 7 Protein interaction network linking ovariectomy to pathogenesis of Alzheimer's disease in mice

3 讨论

女性绝经后雌激素水平下降导致AD患病风险显著增加,而海马是AD发生、发展的关键脑区。本研究在转录组学和蛋白组学整合分析中发现,卵巢切除后雌激素下降主要影响海马轴突生成、细胞骨架动态调节和长链脂肪酸转运。通过与人类海马AD数据库对比,发现OVX后雌激素减少导致海马Aldoc、Etnppl、Itih3和Aldh6a1四个分子表达下降,主要通过影响能量代谢(糖酵解、磷酸乙醇胺分解、氧化还原反应)致使线粒体功能障碍导致能量供应不足以及Itih3介导的细胞外基质重塑,上述途径可能是绝经导致AD的主要原因。

3.1 卵巢雌激素缺乏可能通过影响海马细胞骨架和轴突再生参与AD

本研究通过多组学分析,发现卵巢切除诱导共趋势分子的GO-BP主要富集于轴突发生与支配、KEGG则主要富集于actin细胞骨架调节,强烈提示雌激素缺乏影响actin细胞骨架依赖性轴突生成/消退,参与AD相关学习记忆行为与AD病理,这些发现与AD病理中早期轴突病的既往研究报道一致。比如,有研究报道轴突损伤是AD的早期事件,甚至早于Aβ斑块和神经原纤维缠结的形成[18-19],进行性轴突变性与Aβ沉积和磷酸化tau水平有关[20-21]。本研究发现卵巢切除与海马肌动蛋白聚合-解聚动态高度相关,表明卵巢雌激素的缺乏加剧了海马细胞骨架稳定性丧失,这与前期研究报道[22-24]一致。比如雌激素缺乏不仅下调肌动蛋白聚合相关蛋白的表达、导致F-actin解聚、降低突触密度,还能加速Tau病理和Aβ生成[25-27],而actin聚合的稳定性与学习记忆密切相关[28-30]。这些发现提示,靶向雌激素-细胞骨架-轴突通路对AD的防治具有重要意义。

3.2 卵巢雌激素缺乏可能通过影响海马脂肪酸转运和胞外基质重塑参与AD

既往研究发现,循环中的长链饱和脂肪酸可能与认知功能相关,尤其是二十三烷酸在AD患者海马基因表达中显著变化[31-32],高摄入饱和长链脂肪酸可能通过诱导神经元炎症、增加Aβ生成和磷酸化tau蛋白的表达从而加剧AD病理[33]。膳食中的长链omega-3多不饱和脂肪酸(如DHA)则可通过减少Aβ沉积、抑制神经炎症和改善突触功能延缓AD进展[34-36],较高的DHA水平可降低轻度认知障碍向AD转化的风险[37]。本研究的转录组学和蛋白组学整合共趋势分子的GO富集尤其是GO-分子功能富集分析发现,卵巢切除后会影响海马的长链脂肪酸转运,提示长链脂肪酸转运也可能涉及卵巢雌激素调节海马依赖性AD病理机制的调控。

虽然在共趋势分子的GO和KEGG富集分析中我们并没有发现与胞外基质的富集,但是通过和人类AD海马差异基因数据库的对比,我们发现雌激素缺乏导致海马Itih3基因(参与调节胞外基质透明质酸代谢的主要分子)的变化趋势与人类海马AD数据库的结果一致,这也与AD患者脑脊液中透明质酸的水平变化下降[38-40]的报道一致。已知透明质酸是胞外基质的核心成分,参与了对Aβ聚集和炎性因子释放的调控等重要功能[41-43]、其代谢异常可影响Aβ动态[44,45]。因此,我们的研究结果表明Itih3介导的胞外基质稳定性也是卵巢雌激素调节AD的重要机制之一。

3.3 卵巢雌激素缺乏参与AD相关行为和病理的关键基因

最后,通过和人类海马AD数据库对比与功能分析,在18个共趋势分子中有11个与AD患者海马差异基因数据的表达趋势一致,进一步的蛋白互做网络分析发现*Aldoc*、*Etnppl*、*Aldh6a1*和*Itih3*基因可能是雌激素缺乏导致AD病理的关键基因。*Aldh6a1*和*Aldoc*与糖酵解-线粒体氧化代谢偶联密切相关,*Etnppl*表达于线粒体、与脂质代谢密切相关,*Itih3*基因则是调节胞外基质重要成分透明质酸的关键分子,表明卵巢切除既可破坏海马的能量代谢与抗氧化功能、加剧线粒体

功能障碍与相关的能量代谢障碍,也能通过改变胞外基质的稳定性等多种途径参与AD相关病程和病理。这些结果与线粒体损伤是散发性AD的早期驱动力的结果[46]一致,因为AD早期线粒体电子传递链缺陷促进Aβ沉积及tau磷酸化[46-49],而胞外基质的代谢异常可影响Aβ动态[44-45]。

另外,共趋势中的3个基因(*Prxl2a*、*Set*、*Plin4*)虽然在人类AD数据库中无相关记录,但文献表明*Prxl2a*主要在线粒体中发挥氧化还原酶的作用从而参与AD病理[50];*Set*基因(也称为2PP2A)为编码抑制组蛋白乙酰化酶的多效基因,其表达变化也与AD有关[51];*Plin4*通过线粒体自噬参与AD病理[52]。因此,本研究通过多组学整合分析发现的这3个差异基因,究竟是小鼠和人类的种属差异还是有望成为AD新的检测标志物需要进一步研究。

3.4 小结与展望

综上所述,本研究通过多组学联合分析以及与人类海马AD数据库对比等生物信息学分析方法,发现卵巢雌激素能通过轴突再生、细胞骨架、线粒体能量代谢、胞外基质等多种途径影响AD发生和病理,*Aldoc*、*Etnppl*、*Aldh6a1*和*Itih3*可能是卵巢雌激素发挥上述功能的关键基因,*Prxl2a*、*Set*、*Plin4*则可能是尚未报道过的雌激素调节AD的新靶点基因。上述发现为阐明卵巢雌激素调节AD的发生提供了新的实验证据和潜在的防治靶点,但是这些结果还很初步、尚需要后续实验来进一步证实和挖掘。

利益冲突 本文所有作者声明不存在利益冲突

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