

临床细胞生物学

PPBP基因通过调控滋养细胞融合参与胎儿生长受限的发生

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摘要 胎盘发育异常与胎儿生长受限(fetal growth restriction, FGR)密切相关, 但其发生机制并未被完全阐明。该文利用GEO数据库获得97个正常胎盘样本和81个FGR胎盘样本的基因芯片数据集, 生物信息学分析发现, 差异表达基因(DE-mRNAs)主要富集于趋化因子、HIF1 α 和mTOR等信号通路, 参与细胞炎症、增殖、凋亡和缺氧应答反应等生物过程。PPI网络分析共发现12个关键基因(hub gene)。利用RT-qPCR验证PPI网络分析发现的关键基因(hub基因) *PPBP*、*DUSP1*、*LEP*和*CXCL10*等与生物信息学分析结果一致。FGR胎盘组织病理学检查显示, 与正常胎盘相比, FGR胎盘末端绒毛发育不良、合体体数量增加以及合体化过程受损。进一步的RT-qPCR方法证实, *PPBP*在FGR组胎盘中的表达低于正常组胎盘组织。*PPBP*在弗斯可林诱导BeWo细胞合体化过程中表达上调。干扰*PPBP*后BeWo细胞融合比率和合体化标记物 β -hCG、Syn-1和GCM1的表达以及CREB磷酸化水平下调。研究初步发现, *PPBP*可能通过调控滋养细胞融合分化参与FGR的发生发展。

关键词 胎儿生长受限; 滋养细胞; 合体化; *PPBP*

PPBP Participates in Fetal Growth Restriction through Regulating Trophoblast Fusion

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Abstract Abnormal placental development is associated with FGR (fetal growth restriction). However, the mechanistic bases of FGR have not been clarified. In this study, the mRNA datasets of 97 normal placental samples and 81 FGR placental samples were obtained from GEO database. Bioinformatics analysis of all the data showed that the DE-mRNAs (differentially expressed mRNAs) were mainly clustered in the chemokines, HIF1 α and mTOR

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signaling pathways, and participated in biological processes such as cell inflammation, proliferation, apoptosis and hypoxia response. Further, 12 hub genes were identified by PPI network. RT-qPCR was used to verify the hub genes, including *PPBP*, *DUSP1*, *LEP* and *CXCL10*, and the results were consistent with the bioinformatics analysis. Histopathological investigations revealed hypoplastic distal villous, increased syncytial knots and impaired syncytial layer in FGR placentas compared with normal placentas. Moreover, RT-qPCR confirmed that the expression of *PPBP* in the placenta of the FGR group was lower than that of the normal group. *PPBP* was up-regulated in the process of forskolin-induced fusion in BeWo cells. Knockdown of *PPBP* gene impaired the fusion of BeWo cells by inhibiting the expression of syncytialization markers, β -hCG, Syn-1, GCM1, and CREB phosphorylation. These results suggested that *PPBP* might be involved in the pathogenesis of FGR by inhibiting the fusion of trophoblasts.

Keywords FGR; CTB; syncytialization; *PPBP*

胎儿生长受限 (fetal growth restriction, FGR), 又称宫内生长受限 (intrauterine growth restriction, IUGR), 是常见的妊娠并发症之一。FGR 一般指胎儿未能充分发挥其遗传生长潜能的一种病理状态。临床上一般将超声预估胎儿体重小于同胎龄胎儿应有体重的第3百分位数作为FGR的独立诊断指标, 当超声预估体重仅仅小于第10百分位数时, 需参照其他指标如多普勒血流测速、胎儿生长速度进行综合诊断^[1]。FGR 胎儿通常与较高的死产风险以及成年后发生神经发育障碍、心血管疾病和代谢综合征的风险增加有关^[2-3], 影响全球2.75%~15.53%的新生儿^[4]。FGR的发病机制至今尚未完全阐明, 且临床上FGR一旦发生便无法挽救。因此, 解析FGR的发病机理并寻找合适的医疗干预手段是个严峻的挑战。

目前认为, 胎盘功能异常是FGR发生的重要因素之一。在胎盘形成过程中, 滋养细胞 (cytotrophoblast, CTB) 增殖并分化为绒毛外滋养细胞 (extravillous trophoblast, EVT) 和合体滋养细胞 (syncytiotrophoblast, STB)。CTB 分化为侵袭性表型的 EVT, 可侵入子宫蜕膜和螺旋动脉, 建立组织营养和胎盘血液循环^[5]。CTB 分化融合形成的 STB 是一种能分泌多种激素的多核细胞结构, 是母胎间交换物质的场所和屏障^[5-7]。CTB 分化或其他滋养细胞功能异常可导致螺旋动脉重塑缺陷、胎盘灌注减少和胎盘物质交换障碍, 与FGR等相关并发症发生相关^[8]。因此, 探讨参与胎盘发育异常相关的调控因子对于FGR发生机理的理解尤其重要。

转录组学技术广泛用于识别疾病的潜在调控因子。因此, 本研究采用生物信息学综合分析4

组测序平台的FGR胎盘转录组数据并筛选FGR发生的潜在调控基因。其中亲血小板碱性蛋白 (proplatelet basic protein, PPBP) 又称中性粒细胞激活肽-2 (neutrophil-activating peptide-2, NAP-2), 属于CXC趋化因子家族成员。已经有研究发现, PPBP能诱发细胞中cAMP浓度的上升, 且cAMP是滋养细胞合体化过程中的关键一环^[9-10]。然而, 在滋养细胞中PPBP的表达与细胞合体化之间的联系并不清楚。因此, 本研究利用临床标本和细胞实验探究其生物功能, 旨在为FGR发生的病理基础和前期诊断提供理论依据。

1 材料与方法

1.1 材料

1.1.1 临床样本 本研究得到重庆医科大学伦理委员会批准且标本收集经患者知情同意。人足月胎盘的采集 (孕37~39周; FGR组: $n=6$; 正常组: $n=6$), 所有研究对象均为重庆医科大学附属第一医院选择剖宫产终止妊娠的女性。样本筛选条件: 研究对象有 (但不限于) 妊娠糖尿病、妊娠胆汁淤积症、甲状腺功能不全、妊娠高血压和子痫前期等并发症被排除在病例组和正常组之外。其他排除标准包括先兆流产、胎膜早破、双胞胎或多胎等。FGR诊断标准为产前超声估测胎儿体重低于同胎龄胎儿应有体重第10百分位数且多普勒血流信号异常^[1]。

1.1.2 细胞 BeWo细胞由中国科学院动物研究所王雁玲研究员赠送。

1.1.3 主要试剂 Trizol PrimeScript™ RT Master Mix购自日本TaKaRa公司; SYBR Green Supermix购自美国Bimake公司; 胎牛血清FBS购自德国PAN-

Biotech公司; Lipofectamine 3000转染试剂购自Invitrogen公司; 鼠抗人PPBP抗体购自美国Santa Cruz公司; 兔抗人合胞素1(Syncytin1, Syn-1)抗体购自北京博奥森生物技术有限公司; 鼠抗人胶质细胞缺失因子1(glial cells missing homolog 1, GCM1)抗体、弗斯可林(Forskolin, FSK)和HEPES均购自美国Sigma-Aldrich公司; F12K培养基、小鼠抗人 β -actin抗体、辣根过氧化物酶(horseradish peroxidase, HRP)偶联的山羊抗兔IgG或山羊抗鼠IgG以及山羊抗小鼠TRITC或山羊抗兔FITC二抗均购自武汉博士德生物工程有限公司; 鼠抗人CK7(cytokeratin7)抗体购自北京中杉金桥生物技术有限公司; 鼠抗人E-Cad(E-Cadherin)抗体购自美国CST公司; PPBP-RNAi和阴性对照NC-RNAi购自苏州吉玛基因股份有限公司; 定量引物均由深圳华大基因股份有限公司合成; 蛋白提取及Western blot相关试剂均购自上海碧云天生物技术有限公司; 超敏ECL化学发光试剂盒购自苏州新赛美生物科技有限公司。

1.2 方法

1.2.1 生物信息学分析 从公共基因芯片数据库(gene expression omnibus, GEO)(<http://www.ncbi.nlm.nih.gov/geo/>)获得基于4个测序平台共178例样本(81例FGR胎盘和97例正常胎盘)的mRNA表达谱。使用Bioconductor软件中的limma包(版本3.6.1)和“DESeq2”鉴定差异表达mRNA(differentially expressed mRNAs, DE-mRNAs)。以 $P < 0.05$ 和 $|\log FC| \geq 1$ 为标准鉴别差异表达基因。使用Venny 2.1 web工具检测共有的差异表达基因(<http://bioinfogp.cnb.csic.es/tools/venny/>)。使用Bioconductor软件中的clusterProfile包(版本3.8)进行基因本体(gene ontology, GO)功能注释和京都基因与基因组百科全书(kyoto encyclopedia of genes and genomes, KEGG)通路富集分析。以 $P < 0.05$ 作为富集有统计学意义的指标。使用Bioconductor软件中的ggplot2包(版本3.1.0)绘制前20条显著富集的GO富集分析柱状图和KEGG通路图。利用STING分析工具(<http://stringdb.org/>)构建DE-mRNAs的蛋白-蛋白相互作用(protein-protein interaction, PPI)网络。再使用Cytoscape软件(版本3.6.1)的插件CytoHubba分析网络结构, 通过计算连接度来识别核心差异基因。

1.2.2 样本收集 所有胎盘样本均是在产妇分娩后, 无菌条件下收集的。收集的胎盘样品一部分

立即用福尔马林固定, 用于石蜡包埋; 另一部分, 洗去血液并用液氮速冻后, 置于 -80°C 保存, 用于RT-qPCR。石蜡包埋胎盘组织切成 $5\ \mu\text{m}$ 切片, 进行苏木精-伊红(hematoxylin-eosin, HE)染色或免疫荧光实验。HE染色按照标准程序进行^[11]。

1.2.3 细胞培养 人绒毛膜癌细胞BeWo用含10%的FBS、20 mmol/L HEPES和1%青霉素/链霉素的F12K完全培养基, 于 37°C 、5% CO_2 、20%氧气的恒温培养箱中培养。利用FSK构建BeWo细胞合体化模型。简单来说, 即为 $30\ \mu\text{m}$ 的FSK处理BeWo细胞72 h, 每36 h更换含 $30\ \mu\text{m}$ 的FSK的培养基。用RT-qPCR、Western blot和免疫荧光检测细胞合体化水平。细胞融合比率参照Collett等计算方法^[12], 即算多核(>2 个细胞核)合体与总细胞核的百分比。

1.2.4 细胞转染 将BeWo细胞铺于24孔板内, 待细胞生长密度达到70%~80%根据Lipofectamine 3000说明书分别转染阴性对照(NC-RNAi)和PPBP的小干扰RNA(PPBP-RNAi)进行基因敲低。48 h后获取细胞进行RT-qPCR、Western blot或免疫荧光实验。RT-qPCR验证PPBP-RNAi敲低表达的有效性。PPBP-RNAi和阴性对照的序列见表1。

1.2.5 实时荧光定量PCR(RT-qPCR) Trizol法提取胎盘组织或者细胞的RNA, 逆转为cDNA后用SYBR Green Supermix进行相关基因表达水平的检测。使用 β -actin作为内参基因, 采用 $2^{-\Delta\Delta C_t}$ 进行基因相对表达的计算。相关基因的引物序列见表2。

1.2.6 Western blot 在冰上使用RIPA裂解液提取细胞总蛋白, 取适量的蛋白样品和 $5\times$ SDS-PAGE混合后, 100°C 金属浴加热10 min使蛋白变性。取等量蛋白质用8%的SDS-PAGE进行分离, 以250 mA恒电流转至PVDF膜上后使用5%脱脂奶粉进行封闭, 随后将膜与一抗 4°C 孵育过夜。第2天使用PBST洗涤PVDF膜, 然后与HRP偶联的山羊抗小鼠或兔二抗 37°C 孵育1 h。PBST洗膜后使用ECL显色液激活蛋白条带的化学信号, 化学发光信号的检测和分析分别采用Image Lab 5.2.1和Quantity One 4.6软件。

1.2.7 免疫荧光 ① 组织荧光。将人足月胎盘组织包埋于石蜡中, 并进行 $5\ \mu\text{m}$ 的连续石蜡切片。将组织切片用二甲苯进行脱蜡处理, 梯度酒精中水化后在沸水浴中进行抗原提取。用3%山羊血清和5% BSA进行非特异性结合, 将组织切片与CK7(1:200)和Syn-1(1:200) 4°C 过夜孵育。第2天用PBS洗涤

表1 小干扰RNA序列
Table 1 Sequences of siRNA

小干扰RNA siRNA	序列(5'→3') Sequence (5'→3')
PPBP-RNAi	Sense: GCC UCA GAC UUG AUA CCA CTT Antisense: GUG GUA UCA AGU CUG AGG CTT
NC-RNAi	Sense: UUC UCC GAA CGU GUC ACG UTT Antisense: ACG UGA CAC GUU CGG AGA ATT

表2 引物序列
Table 2 Sequences of primers

基因 Genes	上游引物(5'→3') Forward primers (5'→3')	下游引物(5'→3') Reverse primers (5'→3')
<i>β-actin</i>	AGA TCA TCA GCA ATG CCT CCT	TGG TCA TGA GTC CTT CCA CG
<i>GCM1</i>	GCC AAG CAA GAG CAG CAA A	TCA TCT CAA AGG ACA CAG GTT CA
<i>β-hCG</i>	GTG AAC CCC GTG GTC TCC TA	GGT CAT CAC AGG TCA AGG GG
<i>Syn-1</i>	CCT CAA ACC TCA CCT GTG TAA AAT	AGA GCC ATT CAA ACA ACG ATA GG
<i>SHH</i>	CTG CTG GTA TGC TCG GGA CT	AAA TCG CTC GGA GTT TCT GG
<i>SOD1</i>	AGG GCA TCA TCA ATT TCG AG	TGC CTC TCT TCA TCC TTT GG
<i>CDO1</i>	TCC ATT GGC TTA CAT CGA GTA GA	CCC GAA GTT GCA TTT GGA GT
<i>MAPK8</i>	TCT GGT ATG ATC CTT CTG AAG CA	TCC TCC AAG TCC ATA ACT TCC TT
<i>PPBP</i>	GTA ACA GTG CGA GAC CAC TTC	CTT TGC CTT TCG CCA AGT TTC
<i>PROCR</i>	TGT AGC CAA GAC GCC TCA GAT	AGC AGC GGA TGG TCA GAG G
<i>DUSP1</i>	GAG GGT CAC TAC CAG TAC AAG AGC	GCC TGG CAG TGG ACA AAC A
<i>LEP</i>	TGT GCG GAT TCT TGT GGC T	TAA GGT CAG GAT GGG GTG GA
<i>CXCL10</i>	GTG GCA TTC AAG GAG TAC CTC	TGA TGG CCT TCG ATT CTG GAT T

后与山羊抗小鼠TRITC或山羊抗兔FITC(1:200)于37 °C避光孵育1 h。DAPI(1:10 000)染核10 min。抗荧光淬灭剂封片后通过激光共聚焦显微镜观察图像并拍照。② 细胞荧光。铺于24孔板中的BeWo细胞获取后,用PBS洗涤3次,4%多聚甲醛固定10 min,0.3% Triton X-100通透10 min,5% BSA于37 °C避光封闭30 min,E-Cad(1:1 000)于4 °C孵育过夜。第2天PBS洗涤后与山羊抗小鼠FITC(1:200)于37 °C避光孵育1 h。DAPI(1:10 000)染核10 min。抗荧光淬灭剂封片后通过激光共聚焦显微镜观察图像并拍照。

1.2.8 统计分析 采用Student's *t*或ANOVA检验确定组间数据差异的显著性,数据以均数±标准差($\bar{x}±s$)表示。 $P<0.05$ 为差异有统计学意义。所有的数据分析均通过SPSS 22.0进行。每组数据至少来自3次独立的实验,每个实验重复3次。

2 结果

2.1 FGR胎盘发育相关基因的筛选

本研究从GEO数据共获取了4组FGR足月胎盘

和正常足月胎盘的基因芯片测序数据(GSE24129、GSE35574、GSE100415/GSE75010和GSE114691)(表3)。对所有数据进行差异基因的筛选的结果显示,4组测序数据共有差异基因903个(图1A),其中有6个基因为4组共有的差异基因(表4)。对903个基因进行GO功能注释和KEGG通路富集分析的结果显示,差异基因主要富集于趋化因子、HIF1 α 和mTOR等信号通路(图1B)中并参与细胞炎症、增殖、凋亡和缺氧应答反应等生物过程(图1C)。对差异基因进行PPI蛋白质互作网络分析,节点排名前12的hub基因主要是PPBP、DUSP1、LEP、CXCL10、MAPK8、TLR4、CXCL10、C3AR1、SPII、CCR7、GNG12、GNG2和SOD1。

2.2 RT-qPCR验证影响FGR胎盘发育的潜在调控基因

为了验证生物信息学分析转录组测序结果的准确性和筛选潜在影响FGR胎盘发育的基因,我们前瞻性地采集了FGR组和正常足月胎盘进一步验证(表5)。RT-qPCR分析了部分共有基因和hub基

表3 mRNA数据来源
Table 3 mRNA database sources

GSE序列号	测序平台	组织部位	测序方法	对照组样本数	FGR组样本数
GSE number	Platform	Organization	Sequencing method	Control samples number	FGR samples number
GSE24129	GPL6244	Placenta	mRNA array	8	8
GSE35574	GPL6102	Placenta	mRNA array	40	35
GSE100415/ GSE75010	GPL6244	Placenta	mRNA array	28	20
GSE114691	GPL11154	Placenta	RNA HTC	21	18
Total	/	/	/	97	81

表4 数据库中的共有基因
Table 4 Common genes in database

基因	基因全称	基因功能
Gene	Gene name	Gene function
<i>BHLHE41</i>	Basic helix-loop-helix family member e41	Suppressing MCF-7 cell invasion via MAPK/JNK pathway ^[13] ; promoting the growth of human renal cell carcinoma ^[14] ; regulating alveolar macrophages differentiation ^[15]
<i>PROCR</i>	Endothelial protein c receptor	Inhibiting proinflammatory responses by weakening Th17 activities ^[16] ; promoting hematopoietic stem cell engraftment and expansion ^[17]
<i>SPAG4</i>	Sperm associated antigen 4	Promoting the growth and invasion of renal cancer cells ^[18] ; promoting cell migration in lung carcinoma ^[19]
<i>CDO1</i>	Cysteine dioxygenase type 1	Inactivation of CDO1 improves survival of breast cancer cells ^[20]
<i>DUSP1</i>	Dual specificity phosphatase 1	Regulating tumor cell proliferation, apoptosis and autophagy ^[21-22]
<i>LEP</i>	Leptin	Regulating inflammation, metabolism and immune responses as well as cell proliferation, invasion and apoptosis ^[23-25]

因, 结果与基因芯片分析结果基本一致。与正常组胎盘相比, FGR足月胎盘中*DUSP1*、*LEP*、*SHH*和*PROCO*的表达量较高($P < 0.05$), 而*PPBP*、*CXCL10*和*MAPK8*的表达量较低($P < 0.05$)(图2)。

2.3 FGR胎盘中合体化异常及相关基因PPBP调控滋养细胞合体化的作用

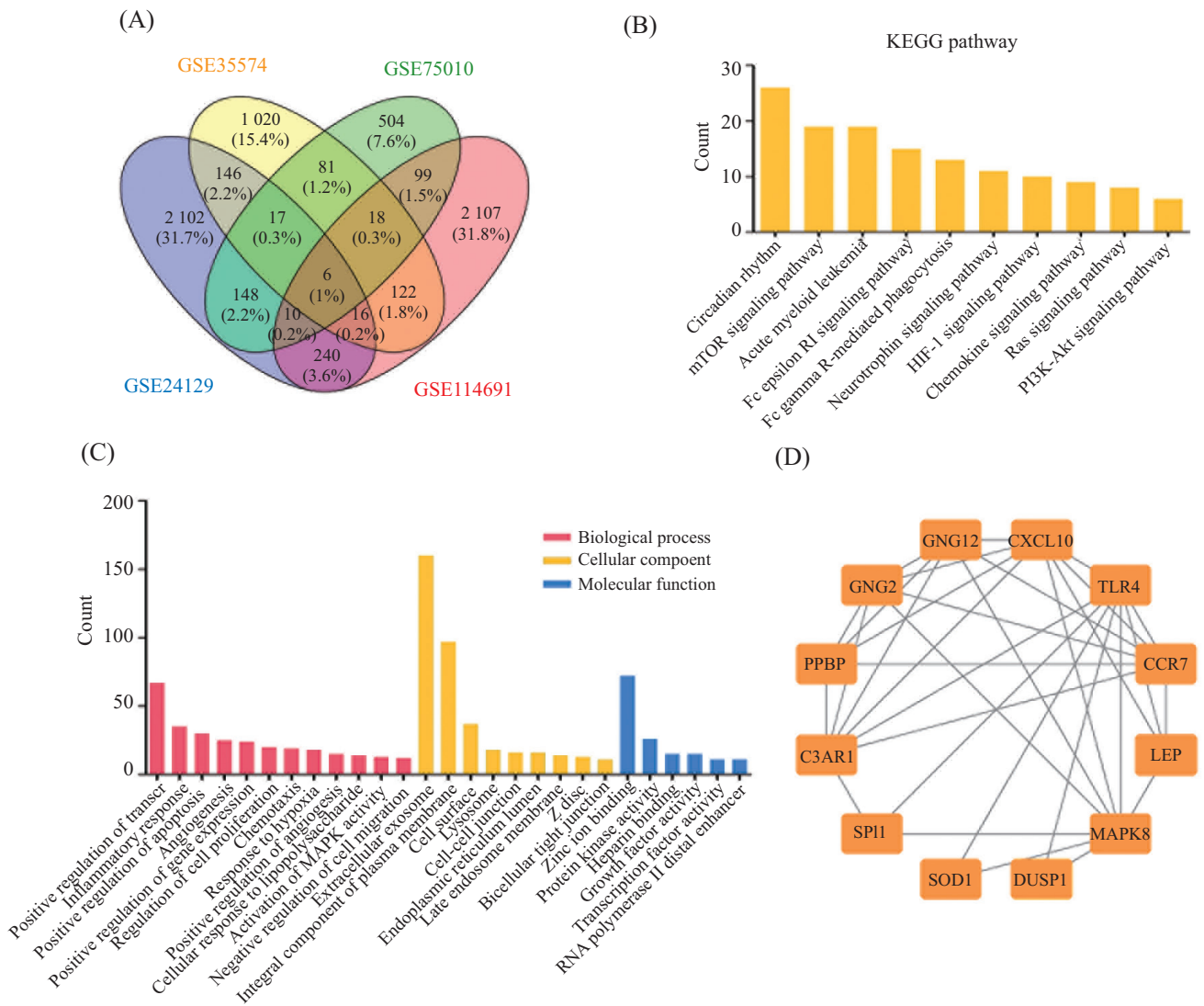
我们对FGR胎盘和正常对照胎盘进行HE和免疫荧光染色。HE结果显示, 正常组胎盘存在更多的末端绒毛细胞和STB, 而FGR组胎盘的合体体则更多($P < 0.05$)(图3A和图3B)。组织免疫荧光染色显示, FGR胎盘中Syn-1阳性的STB较少, 而CK7阳性的CTB较多(图3C)。这些结果均提示, FGR胎盘的合体化过程受损。

结合生物信息学分析和潜在调控基因的验证结果我们选取*PPBP*作为靶基因, 以开展进一步的功能研究。siRNA干扰BeWo细胞中*PPBP*的表达, RT-qPCR和Western blot检测其干扰效果。结果显示, 相比于NC-RNAi组, *PPBP*-RNAi组中*PPBP*的mRNA水

平和蛋白水平均显著下降($P < 0.05$)(图4A和图4B)。干扰*PPBP*后, 如免疫荧光结果所示, 相较于FSK处理组, E-cad表达并未出现显著下调, 且细胞核聚集减少, 细胞融合比率降低(图4C和图4D)($P < 0.05$)。此外通过RT-qPCR和Western blot检测敲低*PPBP*后BeWo细胞的合体化标志基因和蛋白发现, β -人绒毛膜促性腺激素(β -human chorionic gonadotropin, β -hCG)、GCM1和Syn-1在合体化过程中的表达均低于对照组(图4E和图4F)。

2.4 敲低PPBP影响合体化过程中CREB磷酸化水平

为了进一步探究*PPBP*是否通过cAMP通路调控BeWo细胞合体化。我们通过Western blot检测敲低*PPBP*后对于cAMP反应元件结合因子(cAMP-response element binding protein, CREB)的影响, 结果显示在合体化过程中p-CREB水平上调, 但当敲低*PPBP*后p-CREB水平较FSK处理组更低(图5), 提示*PPBP*的敲低对于BeWo细胞合体化过程的抑制作



A: Venn分析4个数据集GSE24129、GSE35574、GSE75010和GSE114691的重叠基因; B: 京都基因与基因组百科全书(KEGG)信号通路富集分析($P < 0.05$); C: 基因本体(GO)功能富集分析($P < 0.05$); D: hub基因蛋白-蛋白相互作用(PPI)网络分析($P < 0.05$)。

A: Venn diagram of DE-mRNAs in the four datasets, GSE24129, GSE35574, GSE75010 and GSE114691; B: kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis results of DE-mRNAs ($P < 0.05$); C: GO function enrichment diagram ($P < 0.05$); D: protein-protein interaction (PPI) network analysis of the hub genes ($P < 0.05$).

图1 生物信息学筛选FGR胎盘发育相关基因

Fig.1 Bioinformatics screening of development-related genes of FGR placenta

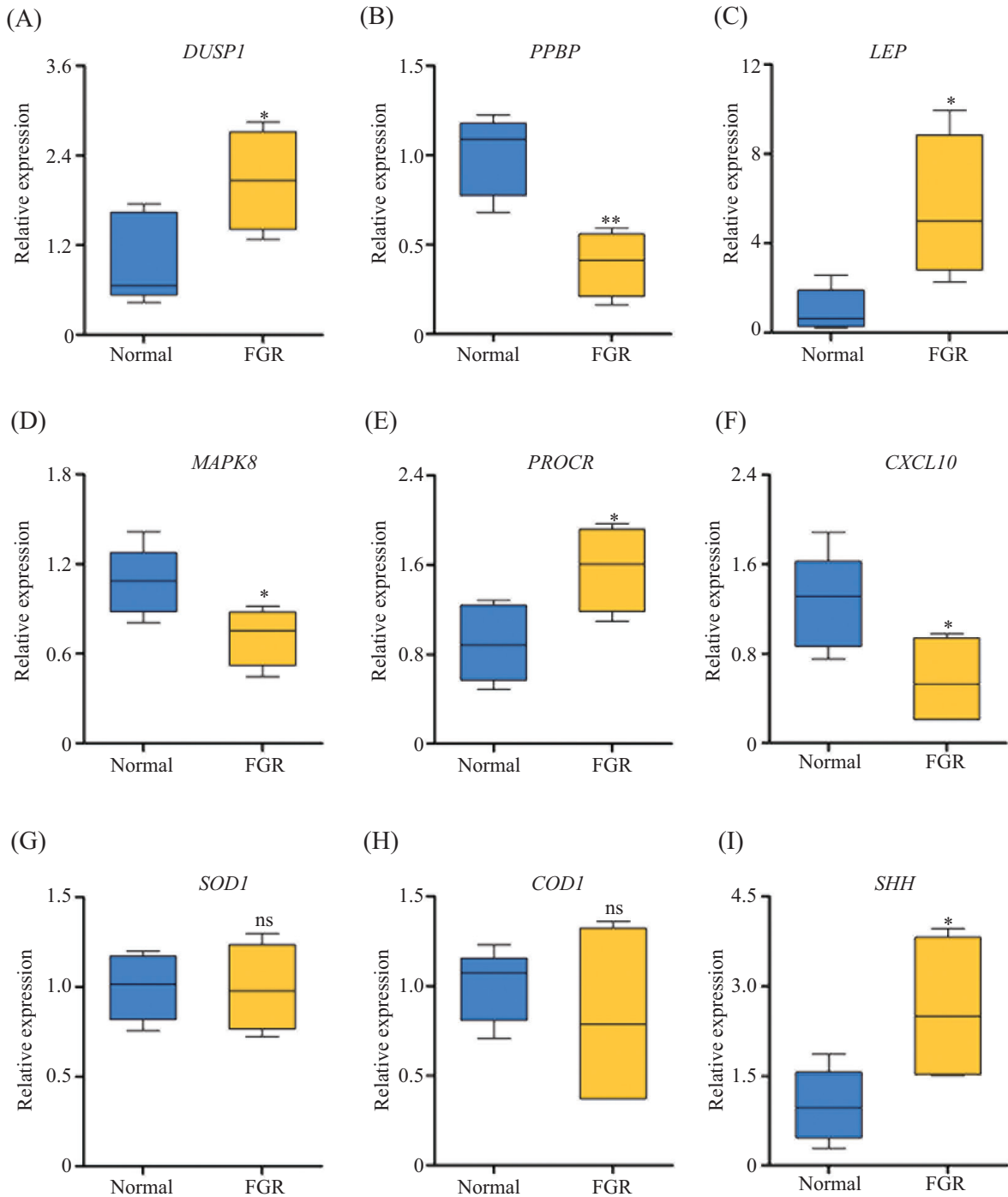
表5 研究样本基本特征

Table 5 Characteristics of the study samples

变量	正常组(n=6)	FGR组(n=6)
Variables	Normal (n=6)	FGR (n=6)
Age /years	30.0±1.6	29.0±1.0
BMI /kg·m ⁻²	20.01±1.18	21.11±3.01
Gestational ages /weeks	39.00±0.55	37.0±1.2*
Body length /cm	50.00±0.63	46.00±2.99*
Neonatal weight /g	3 552.0±468.2	2 249.0±104.8***

* $P < 0.05$, *** $P < 0.001$, 与正常组比较。

* $P < 0.05$, *** $P < 0.001$ vs the Normal group.



ns: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, 与正常组比较。
 ns: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$ vs the Normal group.

图2 验证FGR胎盘的潜在调控基因

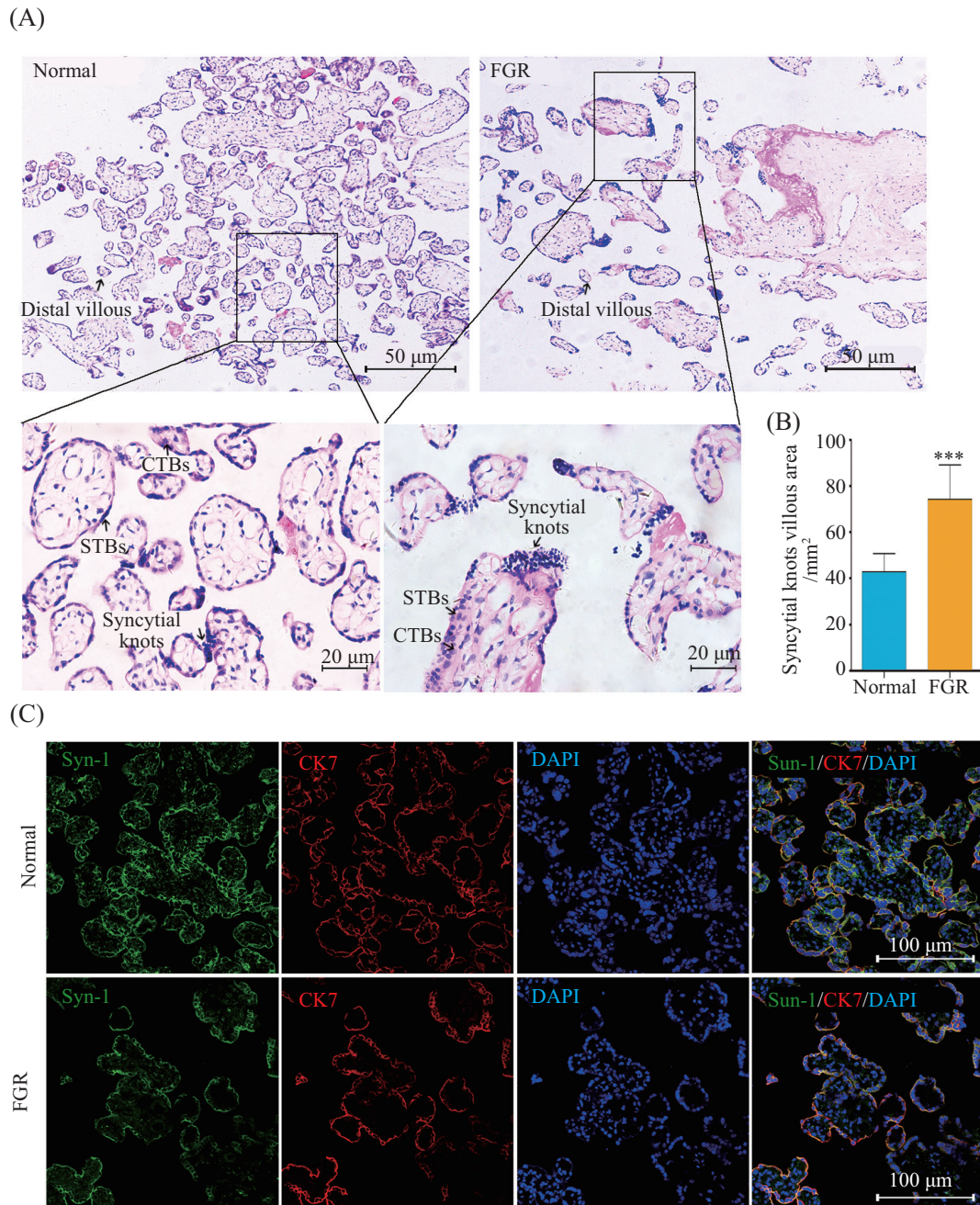
Fig.2 Validation of potential regulatory genes in FGR placentas

用可能是通过影响CREB磷酸化水平,抑制cAMP/CREB通路发挥效应的。

3 讨论

FGR通常是胎盘发育不良和胎盘功能障碍的结

果^[26]。既往研究表明,胎盘结构发育异常与胎儿生长发育迟缓相关^[27-29]。在本研究中,我们观察到FGR胎盘细胞末端绒毛细胞发育缺陷、合体体数量增加及合体化过程受损。同时生物信息学分析结果显示,差异基因主要参与细胞炎症、趋化反应、增殖、调



A: 正常足月胎盘和FGR足月胎盘切片的HE染色, 黑色箭头指向STBs、CTBs、合体体和远端绒毛; B: 正常足月胎盘和FGR足月胎盘中合体体数目, $***P < 0.001$, 与正常组比较; C: 正常和FGR足月胎盘切片的免疫荧光染色。高水平CK7阳性细胞(红色)指示CTB; 低水平CK7阳性细胞(红色)和Syn-1阳性细胞(绿色)表示STB。STBs: 合体滋养层; CTBs: 细胞滋养层。

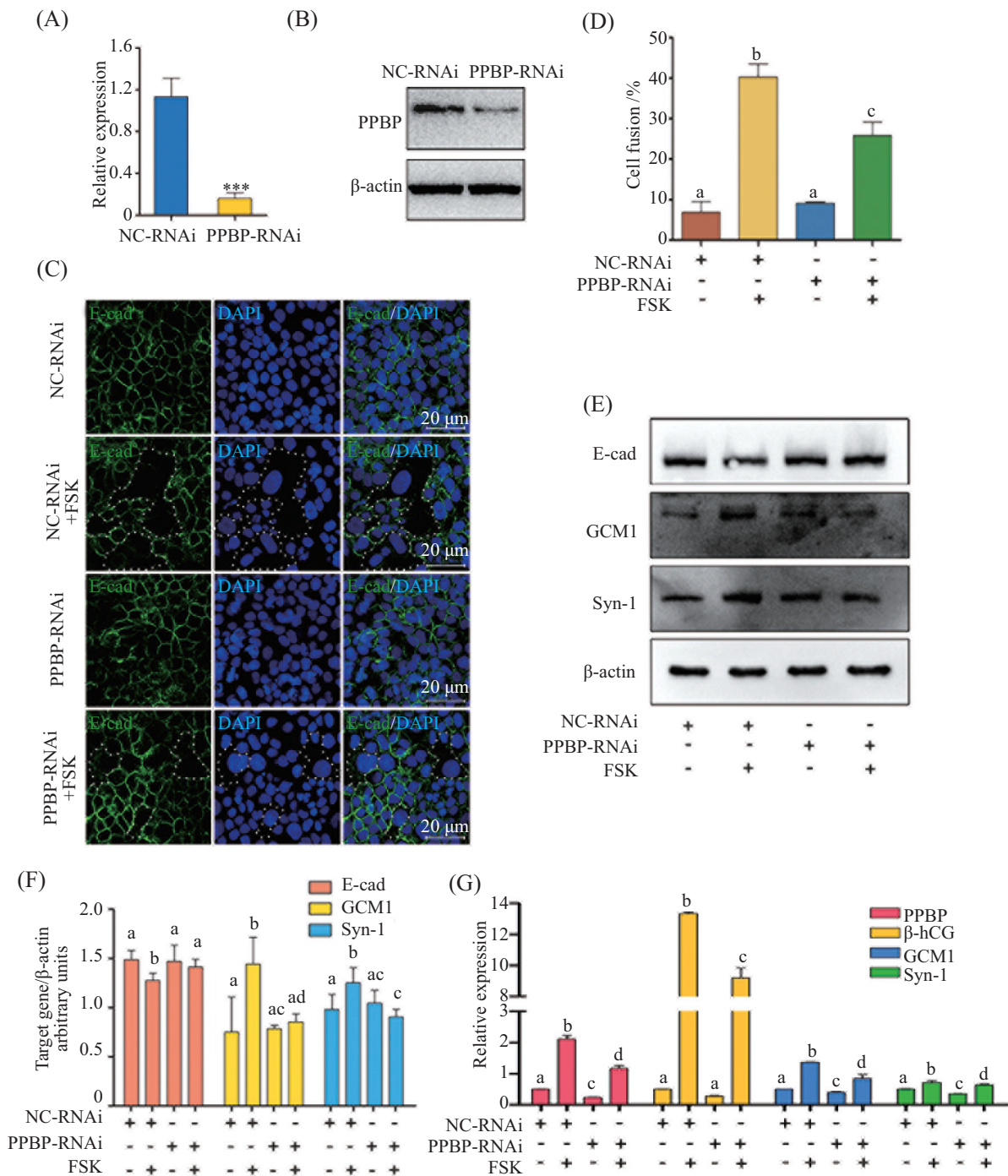
A: HE staining of placental villous sections from normal term pregnancy and FGR; the black arrows point to the STBs, syncytial knots, CTBs and distal villous; B: the number of syncytial knots in normal term pregnancy and FGR, $***P < 0.001$ vs the Normal group; C: immunofluorescence staining of placental villous sections from normal term pregnancy (up) and FGR (down); high CK7-positive cells (red) indicate CTBs; low CK7-positive cells (red) and Syn-1-positive cells (green) indicate the STB. STBs: syncytiotrophoblasts; CTBs: cytotrophoblasts.

图3 FGR胎盘结构异常

Fig.3 Morphological changes in FGR placentas

亡和缺氧应答等生物过程。文献报道, FGR胎盘与正常胎盘的差异表达基因主要涉及血管生成、细胞分化和能量代谢等方面^[29-32]。

胎盘STB细胞是母亲和胎儿之间的屏障, 作为母胎间营养物质和气体交换的场所, 可分泌多种激素以维持妊娠的正常进行。STB合体化过程受损与

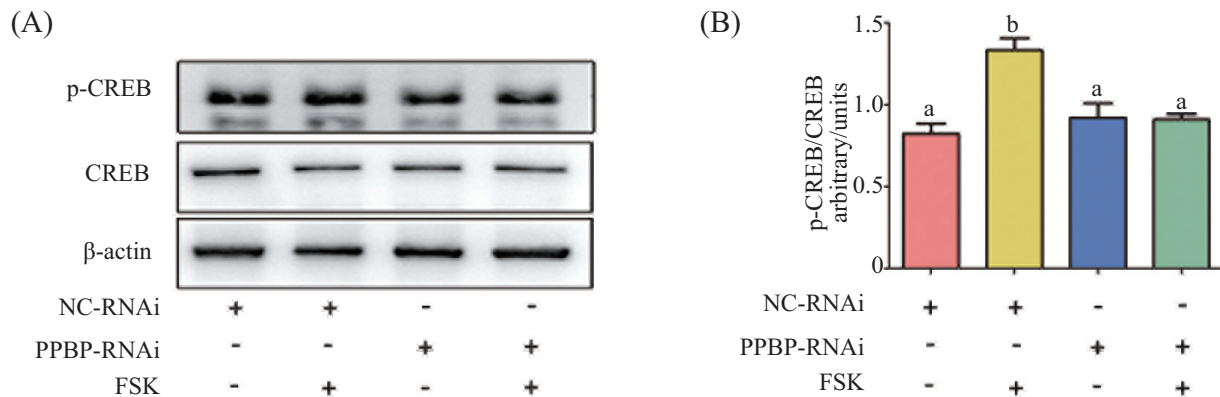


A: RT-qPCR检测NC-RNAi组和PPBP-RNAi组中*PPBP*基因的转录水平; *** $P < 0.001$, 与正常组比较; B: Western blot检测NC-RNAi组和PPBP-RNAi组中*PPBP*的蛋白水平; C、D: 免疫荧光检测BeWo细胞中的E-cad(绿色)水平, 细胞核用DAPI(蓝色)染色, 白色虚线表示融合的BeWo细胞; E、F: Western blot检测合胞化标记物(E-cad、Syn-1和GCM1)的表达水平; G: RT-qPCR检测*PPBP*、 β -hCG、GCM1和Syn-1的相对表达水平。所有统计图中, 标有相同小写字母表示组间“无显著差异”, 不同小写字母表示组间“显著差异”, $P < 0.05$ 。

A: RT-qPCR determination of *PPBP* relative levels in the NC-RNAi group and the PPBP-RNAi group; *** $P < 0.001$ vs the Normal group; B: Western blot determination of *PPBP* protein relative levels in the NC-RNAi group and the PPBP-RNAi group; C,D: immunofluorescence detection of E-cad (green) in BeWo cells; the nuclei are stained with DAPI (blue); the white-dashed lines indicate fused BeWo cells. E,F: Western blot detection of the expression levels of the syncytialization markers (E-cad, Syn-1 and GCM1); G: RT-qPCR determination of the relative levels of *PPBP*, β -hCG, GCM1 and Syn-1. For all variables with letters, the same lowercase letters above the bars indicate “no significant difference”, while different lowercase letters indicate “significant differences” among different groups, $P < 0.05$.

图4 敲低*PPBP*影响BeWo细胞融合

Fig.4 Knockdown of *PPBP* impacts on BeWo cell fusion



A、B: Western blot检测CREB和p-CREB的表达水平。标有相同小写字母表示组间“无显著差异”,不同小写字母表示组间“显著差异”, $P<0.05$ 。

A,B: Western blot detection the expression levels of the CREB and p-CREB. The same lowercase letters above the bars indicate “no significant difference”, while different lowercase letters indicate “significant differences” among different groups, $P<0.05$.

图5 敲低PPBP对BeWo细胞融合过程中CREB磷酸化水平的影响

Fig.5 The effect of knockdown of PPBP on the phosphorylation level of CREB during BeWo cells fusion

FGR的发生密切相关^[6,33-34]。在本研究中,我们发现,FGR胎盘中PPBP的表达降低。同时,PPBP基因的敲低也能够抑制BeWo细胞的融合。已有报道显示,PPBP能诱发中性粒细胞中环磷腺苷效应元件结合蛋白cAMP的浓度的升高^[10]。cAMP信号通路是参与滋养细胞融合过程中极其重要的途径之一。cAMP胞内浓度的增加可以触发CREB蛋白,使其磷酸化水平增加,进而诱导其下游细胞融合相关基因的表达,如 β -hCG、GCM1和Syn-1等^[9]。

我们发现,PPBP的下调有效地抑制了FSK处理后BeWo细胞中CREB的磷酸化水平。这表明,PPBP可能通过调控cAMP/CREB信号通路影响BeWo细胞的合体化过程。此外,PPBP也被发现能刺激前列腺素E2分泌^[35],细胞内前列腺素E2的增加能够抑制HTR-8/SVneo细胞的增殖^[36],且细胞的增殖与细胞周期密切相关,同时细胞融合往往发生在细胞周期阻滞之前^[37-38]。因此,PPBP是否可能通过影响细胞周期来调控细胞融合,值得进一步研究。

在本研究中,我们还挖掘到其他可能参与FGR发生的潜在调控基因如:LEP、CXCL10、DUSP1、MAPK8等。其中能够调节胎盘营养物质转运的蛋白的瘦素(leptin, LEP)的表达失调与先兆子痫和FGR等妊娠期相关并发症的发生有关^[24,39]。此外,子痫前期的高瘦素血症可能是一种代偿机制,促进营养物质通过灌注不良的胎盘血脑屏障运输^[24]。双特异性磷酸酶1(dual specificity phosphatase 1, DUSP1)可抑制肿

瘤细胞增殖、迁移和侵袭^[40]。由于EVT细胞侵袭入母体子宫与肿瘤细胞的浸润转移具有相似性,因此,DUSP1是否能影响EVT细胞的这些功能从而影响胎盘的发育还不清楚。

本研究中,我们对生物信息学分析筛选的FGR的部分相关基因进行了相关验证,尚有多个基因亦可能参与滋养细胞功能的调控或参与胎盘相关疾病的发生。进一步的研究将有助于深入理解FGR等胎盘源性疾病的发病机制。

参考文献 (References)

- [1] 中华医学会围产医学分会胎儿医学学组, 中华医学会妇产科学分会产科学组. 胎儿生长受限专家共识, 2019版[J]. 中华围产医学杂志(FETAL MEDICINE SUBGROUP, SOCIETY OF PERINATAL MEDICINE, CHINESE MEDICAL ASSOCIATION OBSTETRICS SUBGROUP, SOCIETY OF OBSTETRICS AND GYNECOLOGY, CHINESE MEDICAL ASSOCIATION. Expert consensus on fetal growth restriction, 2019 [J]. Chin J Perinat Med), 2019, 22(6): 361-80.
- [2] LEES C C, MARLOW N, VAN WASSENAER-LEEMHUIS A, et al. 2 year neurodevelopmental and intermediate perinatal outcomes in infants with very preterm fetal growth restriction (TRUFFLE): a randomised trial [J]. Lancet, 2015, 385(9983): 2162-72.
- [3] CURETON N, KOROTKOVA I, BAKER B, et al. Selective targeting of a novel vasodilator to the uterine vasculature to treat impaired uteroplacental perfusion in pregnancy [J]. Theranostics, 2017, 7(15): 3715-31.
- [4] LIU J, WANG X F, WANG Y, et al. The incidence rate, high-risk factors, and short- and long-term adverse outcomes of fetal growth restriction: a report from mainland China [J]. Medicine

- (Baltimore), 2014, 93(27): e210.
- [5] ADU-GYAMFI E A, CZIKA A, GORLEKU P N, et al. The involvement of cell adhesion molecules, tight junctions, and gap junctions in human placentation [J]. *Reprod Sci*, 2021, 28(2): 305-20.
- [6] CHANG C W, WAKELAND A K, PARAST M M. Trophoblast lineage specification, differentiation and their regulation by oxygen tension [J]. *J Endocrinol*, 2018, 236(1): R43-56.
- [7] KNOFLER M, HAIDER S, SALEH L, et al. Human placenta and trophoblast development: key molecular mechanisms and model systems [J]. *Cell Mol Life Sci*, 2019, 76(18): 3479-96.
- [8] LYALL F, ROBSON S C, BULMER J N. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome [J]. *Hypertension*, 2013, 62(6): 1046-54.
- [9] GERBAUD P, TASKEN K, PIDOUX G. Spatiotemporal regulation of cAMP signaling controls the human trophoblast fusion [J]. *Front Pharmacol*, 2015, 6: 202.
- [10] BRANDT E, PETERSEN F, FLAD H D. Recombinant tumor necrosis factor- α potentiates neutrophil degranulation in response to host defense cytokines neutrophil-activating peptide 2 and IL-8 by modulating intracellular cyclic AMP levels [J]. *J Immunol*, 1992, 149(4): 1356-64.
- [11] SCHMITZ N, LAVERTY S, KRAUS V B, et al. Basic methods in histopathology of joint tissues [J]. *Osteoarthritis Cartilage*, 2010, 18(Suppl 3): S113-6.
- [12] COLLETT G P, LINTON E A, REDMAN C W, et al. Downregulation of caveolin-1 enhances fusion of human BeWo choriocarcinoma cells [J]. *PLoS One*, 2010, 5(5): e10529.
- [13] ZHANG D, ZHENG Q, WANG C, et al. BHLHE41 suppresses MCF-7 cell invasion via MAPK/JNK pathway [J]. *J Cell Mol Med*, 2020, 24(7): 4001-10.
- [14] SHEN Z, ZHU L, ZHANG C, et al. Overexpression of BHLHE41, correlated with DNA hypomethylation in 3'UTR region, promotes the growth of human clear cell renal cell carcinoma [J]. *Oncol Rep*, 2019, 41(4): 2137-47.
- [15] RAUSCHMEIER R, GUSTAFSSON C, REINHARDT A, et al. Bhlhe40 and Bhlhe41 transcription factors regulate alveolar macrophage self-renewal and identity [J]. *EMBO J*, 2019, 38(19): e101233.
- [16] KISHI Y, KONDO T, XIAO S, et al. Protein C receptor (PROCR) is a negative regulator of Th17 pathogenicity [J]. *J Exp Med*, 2016, 213(11): 2489-501.
- [17] KOHLSCHEEN S, SCHENK F, ROMMEL M G E, et al. Endothelial protein C receptor supports hematopoietic stem cell engraftment and expansion in Mpl-deficient mice [J]. *Blood*, 2019, 133(13): 1465-78.
- [18] KNAUP K X, MONTI J, HACKENBECK T, et al. Hypoxia regulates the sperm associated antigen 4 (SPAG4) via HIF, which is expressed in renal clear cell carcinoma and promotes migration and invasion *in vitro* [J]. *Mol Carcinog*, 2014, 53(12): 970-8.
- [19] JI Y, JIANG J, HUANG L, et al. Sperm-associated antigen 4 (SPAG4) as a new cancer marker interacts with Nesprin3 to regulate cell migration in lung carcinoma [J]. *Oncol Rep*, 2018, 40(2): 783-92.
- [20] JESCHKE J, O'HAGAN H M, ZHANG W, et al. Frequent inactivation of cysteine dioxygenase type 1 contributes to survival of breast cancer cells and resistance to anthracyclines [J]. *Clin Cancer Res*, 2013, 19(12): 3201-11.
- [21] WANG J, ZHOU J Y, KHO D, et al. Role for DUSP1 (dual-specificity protein phosphatase 1) in the regulation of autophagy [J]. *Autophagy*, 2016, 12(10): 1791-803.
- [22] YANG J, SUN L, HAN J, et al. DUSP1/MKP-1 regulates proliferation and apoptosis in keratinocytes through the ERK/Elk-1/Egr-1 signaling pathway [J]. *Life Sci*, 2019, 223: 47-53.
- [23] ABELLA V, SCOTECE M, CONDE J, et al. Leptin in the interplay of inflammation, metabolism and immune system disorders [J]. *Nat Rev Rheumatol*, 2017, 13(2): 100-9.
- [24] PEREZ-PEREZ A, TORO A, VILARINO-GARCIA T, et al. Leptin action in normal and pathological pregnancies [J]. *J Cell Mol Med*, 2018, 22(2): 716-27.
- [25] GHASEMI A, SAEIDI J, AZIMI-NEJAD M, et al. Leptin-induced signaling pathways in cancer cell migration and invasion [J]. *Cell Oncol (Dordr)*, 2019, 42(3): 243-60.
- [26] BURTON G J, JAUNIAUX E. Pathophysiology of placental-derived fetal growth restriction [J]. *Am J Obstet Gynecol*, 2018, 218(2S): S745-61.
- [27] HUTCHEON J A, MCNAMARA H, PLATT R W, et al. Placental weight for gestational age and adverse perinatal outcomes [J]. *Obstet Gynecol*, 2012, 119(6): 1251-8.
- [28] MAYHEW T M, OHADIKE C, BAKER P N, et al. Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction [J]. *Placenta*, 2003, 24(2/3): 219-26.
- [29] GUO L, TSAI S Q, HARDISON N E, et al. Differentially expressed microRNAs and affected biological pathways revealed by modulated modularity clustering (MMC) analysis of human preeclamptic and IUGR placentas [J]. *Placenta*, 2013, 34(7): 599-605.
- [30] STRUWE E, BERZL G, SCHILD R, et al. Microarray analysis of placental tissue in intrauterine growth restriction [J]. *Clin Endocrinol (Oxf)*, 2010, 72(2): 241-7.
- [31] NISHIZAWA H, OTA S, SUZUKI M, et al. Comparative gene expression profiling of placentas from patients with severe preeclampsia and unexplained fetal growth restriction [J]. *Reprod Biol Endocrinol*, 2011, 9: 107.
- [32] GIBBS I, LEAVEY K, BENTON S J, et al. Placental transcriptional and histologic subtypes of normotensive fetal growth restriction are comparable to preeclampsia [J]. *Am J Obstet Gynecol*, 2019, 220(1): 110e1- e21.
- [33] DAAAYANA S, BAKER P, CROCKER I. An image analysis technique for the investigation of variations in placental morphology in pregnancies complicated by preeclampsia with and without intrauterine growth restriction [J]. *J Soc Gynecol Investig*, 2004, 11(8): 545-52.
- [34] HEAZELL A E, MOLL S J, JONES C J, et al. Formation of syncytial knots is increased by hyperoxia, hypoxia and reactive oxygen species [J]. *Placenta*, 2007, 28(Suppl A): S33-40.
- [35] CASTOR C W, MILLER J W, WALZ D A. Structural and biological characteristics of connective tissue activating peptide (CTAP-III), a major human platelet-derived growth factor [J]. *Proc Natl Acad Sci USA*, 1983, 80(3): 765-9.
- [36] BIONDI C, FERRETTI M E, PAVAN B, et al. Prostaglandin E2 inhibits proliferation and migration of HTR-8/SVneo cells, a

- human trophoblast-derived cell line [J]. *Placenta*, 2006, 27(6/7): 592-601.
- [37] LU X, WANG R, ZHU C, et al. Fine-tuned and cell-cycle-restricted expression of fusogenic protein syncytin-2 maintains functional placental syncytia [J]. *Cell Rep*, 2017, 21(5): 1150-9.
- [38] HARDWICK L J A, AZZARELLI R, PHILPOTT A. Cell cycle-dependent phosphorylation and regulation of cellular differentiation [J]. *Biochem Soc Trans*, 2018, 46(5): 1083-91.
- [39] MIEHLE K, STEPAN H, FASSHAUER M. Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia [J]. *Clin Endocrinol (Oxf)*, 2012, 76(1): 2-11.
- [40] SHEN J, ZHANG Y, YU H, et al. Role of DUSP1/MKP1 in tumorigenesis, tumor progression and therapy [J]. *Cancer Med*, 2016, 5(8): 2061-8.