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·基础研究·

CircPDS5B水平上调参与早产的病理生理机制研究

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[摘 要] **目的** 探讨CircPDS5B水平上调参与早产的病理生理分子机制。**方法** 收集足月儿(Group 1,孕周≥37周)、晚期早产儿(Group 2,34周≤孕周<37周)、早产儿(Group 3,32周<孕周<34周)和极早产儿(Group 4,孕周≤32周)的胎盘组织,每组5例样本。提取其总RNA并进行CircRNAs微阵列分析。qPCR法验证胎盘组织中前10位显著上调和前10位显著下调的CircRNAs的表达水平。在体外人胎盘微血管内皮细胞(hPMEC)中采用RNA免疫共沉淀法验证CircPDS5B与异质核糖核蛋白L(hnRNPL)及hnRNPL与缺氧诱导因子-1α(HIF-1α) mRNA的直接结合关系。将hPMEC分为Control组、Lenti-sh-CircPDS5B组、Lenti-sh-NT组、Lenti-OE-CircPDS5B组及Lenti-OE-Vector组,其中Control组细胞未进行转染,其余组细胞分别转染相应的慢病毒颗粒。Western blot法测定各组细胞中HIF-1α和血管内皮生长因子(VEGF)-A蛋白表达水平。**结果** CircRNAs微阵列分析和qPCR法验证结果显示,与Group 1相比,CircPDS5B在Group 4中上调最显著($P<0.05$)。RNA免疫共沉淀结果显示,CircPDS5B与hnRNPL以及hnRNPL与HIF-1α mRNA存在直接结合关系。与Lenti-sh-NT组相比,Lenti-sh-CircPDS5B组细胞中HIF-1α和VEGF-A的表达水平升高($P<0.05$)。与Lenti-OE-Vector组相比,Lenti-OE-CircPDS5B组细胞中HIF-1α和VEGF-A的表达水平降低($P<0.05$)。**结论** CircPDS5B水平上调通过hnRNPL结合和隔离HIF-1α mRNA抑制VEGF表达参与早产的病理生理机制。

[关键词] 早产;CircRNAs表达谱;CircPDS5B;HIF-1α;VEGF-A

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Up-regulation of CircPDS5B level involving in the pathophysiological mechanism of premature birth

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Abstract: Objective To explore the pathophysiological molecular mechanism of the up-regulation of CircPDS5B level involved in

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premature birth. **Methods** The placental tissues of full-term infants (Group 1, gestational age ≥ 37 weeks), late premature infants (Group 2, 34 weeks ≤ gestational age < 37 weeks), premature infants (Group 3, 32 weeks < gestational age < 34 weeks) and very premature infants (Group 4, gestational age ≤ 32 weeks) were collected, with 5 samples in each group. Total RNA was extracted from placental tissues for CircRNAs microarray. The expression levels of the top 10 significantly up-regulated and top 10 significantly down-regulated CircRNAs in the placental tissues were verified by qPCR. The direct binding relationship between CircPDS5B and heterogenous nuclear ribonucleoprotein L (hnRNPL), as well as hnRNPL and hypoxia-induced factor-1α (HIF-1α) mRNA were verified by RNA immunocoprecipitation in human placental microvascular endothelial cells (hPMEC) *in vitro*. HPMEC were divided into Control group, Lenti-sh-CircPDS5B group, Lenti-sh-NT group, Lenti-OE-CircPDS5B group and Lenti-OE-Vector group. The cells in the Control group were not transfected, and the cells in the other groups were transfected with corresponding lentivirus particles respectively. The expression levels of HIF-1α and vascular endothelial growth factor (VEGF)-A protein of cells in each group were determined by Western blot. **Results** The results of CircRNAs microarray analysis and qPCR verification showed that compared with Group 1, CircPDS5B was up-regulated most significantly in Group 4 (P<0.05). The RNA immunoprecipitation results showed that there was a direct binding relationship between CircPDS5B and hnRNPL, as well as hnRNPL and HIF-1α mRNA. Compared with Lenti-sh-NT group, the expression levels of HIF-1α and VEGF-A of cells in Lenti-sh-CircPDS5B group were increased (P<0.05). Compared with Lenti-OE-Vector group, the expression levels of HIF-1α and VEGF-A of cells in Lenti-OE-CircPDS5B group were decreased (P<0.05). **Conclusion** Up-regulation of CircPDS5B level is involved in the pathophysiological mechanisms of premature birth by inhibiting VEGF expression through hnRNPL binding and sequestering HIF-1α mRNA.

Keywords: premature birth; CircRNAs microarray; CircPDS5B; HIF-1α; VEGF-A

早产儿是指在妊娠37周之前出生,或从妇女末次月经的第1天起妊娠少于259 d出生的婴儿[1]。全球范围内每年有1 500万早产儿,发生率为11.1%,其中约70%为自然早产,30%为人为引导的早产[2]。引发自然早产的原因有:出现子痫前期、孕前和妊娠糖尿病、宫颈功能不全、贫血等早产相关并发症;或是胎盘、子宫或胎儿状况(包括胎盘早剥、前置胎盘、羊水过多、子宫异常、平滑肌瘤和胎儿出生缺陷等)不佳等[3]。

胎盘血管生成异常和胎盘缺氧参与子痫前期和胎儿早产的病理生理机制。血管内皮生长因子(vascular endothelial growth factor, VEGF)是胎盘血管生成的关键调节因子。有研究发现孕24周、28周和32周母体循环内分泌腺源-VEGF水平升高是一种自然早产预测因子[4]。子痫前期孕妇胎盘母体中央区、胎儿中央区、母体外周区和胎儿外周区的VEGF水平均低于健康对照组[5]。说明母体和/或胎盘VEGF水平异常可能参与子痫前期和胎儿早产。

CircRNAs是一类通过mRNA前体反向剪接产生的新型内源性非编码RNA,广泛分布于体内,具有调控基因转录、翻译的能力以及很强的组织和细胞类型特异性[1-2]。由于CircRNAs是一类共价闭合环状结构的RNA分子,相对于其对应的线性RNA分子具有更高的稳定性和抵抗核酸酶降解的能力。有研究表明,CircRNAs通过多种机制参与复杂多样的生物学过程,包括作为miRNA海绵体、与RNA结合蛋白(RNA binding proteins, RBPs)相互作用调节mRNA的表达水平[6]。初乳外泌体中的CircRNAs通过VEGF信号通路参与调控人类足月儿和早产儿的肠道发育过程[7]。早产儿血清中存在一系列异常CircRNAs表达谱,可能在早产儿支气管肺发育不良的发病机制中起重要作用[8]。孕妇胎盘等组织VEGF水平降低是否存在CircRNAs水平和表达调控异常,是本次研究拟探讨的重点内容。本研究通过提取早产儿与足月儿的总RNA并进行CircRNAs微阵列分析和相关分子机制验证实验,以期深入探讨胎盘缺氧通过CircRNAs介导子痫前期和胎儿早产的病理生理机制。

1 材料与方法

1.1 实验材料

人胎盘微血管内皮细胞(human placental microvascular endothelial cells, hPMEC)购自武汉普诺赛生物科技有限公司。RNA Easy Fast动物组织/细胞总RNA提取试剂盒(批号D145451)、FastKing cDNA第一链合成试剂盒(去基因组,批号2511679)、SuperReal荧光定量预混试剂增强版(SYBR Green,批号2052545)购自天根生化科技(北京)有限公司。Arraystar Super RNA Labeling Kit(批号AS-1187-2820)和Arraystar Human circRNA Array(8×15 K,批号AS-3570-4812)购自美国Arraystar。EZMagna RNA免疫共沉淀(RNA immunoprecipitation,RIP)试剂盒(批号17-701)购自美国Millipore。一抗抗体兔抗人缺氧诱导因子-1α(hypoxia-induced factor-1α,HIF-1α;批号35112)、兔抗人VEGF-A(批号29154)、兔抗人GAPDH(批号38431)购自美国Thermo Fisher Scientific。山羊抗兔HRP-标记IgG二抗(批号X97153)购自上海信帆生物科技有限公司。所有引物委托生工生物工程(上海)股份有限公司代为设计和合成。委托和元生物技术(上海)股份有限公司完成慢病毒颗粒的包装实验。

1.2 临床样本采集

收集2021年1月至2022年1月于我院进行产检和生产的孕妇胎盘组织，包括足月儿（Group 1，孕周≥37周）、晚期早产儿（Group 2，34周≤孕周<37周）、早产儿（Group 3，32周<孕周<34周）和极早产儿（Group 4，孕周≤32周）的胎盘组织，每组5例样本。足月儿胎盘组织纳入标准：妊娠满37周后分娩的新生儿胎盘组织。早产儿胎盘组织纳入标准：妊娠<37周，未通过药物终止妊娠的新生儿胎盘组织。排除标准：多胎妊娠；巨大儿；未足月胎膜早破；瘢痕子宫；胎盘早剥或前置；羊水过少；妊娠期糖尿病；妊娠期内胆汁淤积症；羊水穿刺检查致早产；因外伤或感染致早产。胎盘组织取出后立即剪成小块，加入RNA保护液，送实验室提取总RNA，或冻存于-80 ℃冰箱备用。所有人类胎盘组织样本的采集和使用均通过我院伦理委员会的批准（批准号：2022-340），并经产妇本人知情同意。

1.3 人胎盘微血管内皮细胞培养

hPMEC培养于含10%胎牛血清的完全内皮细胞培养液中，置于含5%CO_2的37 ℃恒温湿润的细胞孵育箱中维持培养。

1.4 CircRNAs微阵列分析

使用RNA Easy Fast动物组织/细胞总RNA提取试剂盒提取Group 1~4组织总RNA。用RNase R酶切总RNA以去除线性RNA并富集CircRNAs。利用随机引物法扩增富集的CircRNAs，转录成荧光RNA（Arraystar Super RNA Labeling Kit）并杂交到Arraystar Human circRNA Array（8×15 K）上。结束后进行短暂洗涤，用安捷伦扫描仪G2505C扫描阵列。使用Agilent Feature Extraction软件（v11.0.1.1）对采集到的阵列图像进行分析。原始表达强度进行log2变换并归一化。组间差异分析采用*t*检验。截断值为$P\leq0.05$，$|FC|\geq2.0$。假设每个基因的样本间原始表达强度经log2转换和归一化后强度值为正态分布。本研究数据集中>90%的基因强度值经*Shapiro-Wilk*正态性检验具备正态性分布特征。

1.5 qPCR

向每100 mg胎盘组织中加入100 μL TRIzol裂解液，在液氮中裂解组织。使用RNA Easy Fast动物组织/细胞总RNA提取试剂盒提取总RNA。使用FastKing cDNA第一链合成试剂盒（去基因组）反转录合成cDNA。使用SuperReal荧光定量预混试剂增强版（SYBR Green）进行qPCR扩增。引物序列见表1。

表1 引物序列

基因	正向	反向
CircPDS5B	5′-ACTTTCATGGACATGGACCAG-3′	5′-GCTCCTGAGGCTCCTTAC-3′
CircPostn	5′-GCGCGGGCGTGCGAGCAGCG-3′	5′-CCTCCCCCTTGGGATCCCGC-3′
CircTlk1	5′-GCTTGGGATCTTTAAGTGAC-3′	5′-GAAGAAAGAGAAAAGCAGA-3′
CircCrim1	5′-AGCAGCCACCGCCGCCGCCG-3′	5′-CTCCCGGGAGGAGGAGGAG-3′
CircZfpm2	5′-GCTTGCTCATCTCCGAACG-3′	5′-GGCCGAGCCTGGCCAGCGGC-3′
CircElf2	5′-AGAATTCATCCATGCTGCTA-3′	5′-GAACCCATGGATACCTCTCC-3′
CircRunx2	5′-CAGCAAAACTTCTTTTGGG-3′	5′-GACGTGAGCCCGGTGGTGGC-3′
CircCreb1	5′-CGTGGGGGAGAGAATAAAA-3′	5′-CTAAATGACCATGGAATCTGG-3′
CircSnx5	5′-GTTACAAGGCAACATGAAG-3′	5′-GCTCCTACGAAGCCCGAC-3′
CircGm20721	5′-CCGGTACTGCAGACGAAGTG-3′	5′-GATCGAGTCCCCAGACGAC-3′
CircRrbp1	5′-GTCCTGGAGGTGCCATTTCCC-3′	5′-GACACTCAAACCTTGGGGG-3′
CircTrappc11	5′-CTAACGGGCCTGGATGTAG-3′	5′-GGAGAGCTGATCGAGTACCA-3′
CircMcu	5′-GCCGGCTGCTTCCCTGGGC-3′	5′-CTGGCAGAATTTGGGAGCTG-3′
CircGarnl3	5′-CCCTTTCTTCTTGTCCGTG-3′	5′-CAAATATTTTTTAGGACAAG-3′
CircEpc1	5′-GATGAGTAAACTGTCGTTTC-3′	5′-GAGGATCTGCCCGACCTGC-3′
CircSenp6	5′-GCCTGACGGTCGCAGAGACC-3′	5′-GTTTGTGTGCTCGCTTTCA-3′
CircBnc2	5′-CTGGTAAACTGCACATGTG-3′	5′-GCACATGCCTTGGATAAGC-3′
CircXylt1	5′-GCGGCGCCGTGCGCCCGGAG-3′	5′-GTCGTGTGGAATTTCAGCAG-3′
CircTcf4	5′-GCTAAAATGCATCACCAACA-3′	5′-GTTTTCACCTCCTGTGAGC-3′
CircAsxl3	5′-GTGCGGGTCACCGGGTCAC-3′	5′-CAGAACCATCAATGAGATGC-3′
U6	5′-GCTTCGGCAGCACATATACTAAAAT-3′	5′-CGCTTCACGAATTTGCGTGTCAT-3′
HIF-1α	5′-CTGAAGTTTTTTATGAGCT-3′	5′-GCTTACCATCAGCTATTTGC-3′

1.6 RNA免疫共沉淀

使用EZMagna RIP试剂盒按照说明书进行RIP检测。使用含蛋白酶和RNase抑制剂的RIP裂解液裂解hPMEC（裂解总细胞数3×10^7个）。然后，将RIP裂解物与抗异质核糖核蛋白L（heterogenous nuclear ribonucleoprotein L，hnRNPL）抗体（hnRNPL组）或抗IgG抗体（IgG组）一起孵育（每50 μL磁珠悬液含2 μg抗体，4 ℃共同孵育2 h）。采用qPCR分析洗脱后的共沉淀RNA。

1.7 Western blot

向6孔板的细胞培养物中加入1 mL含蛋白酶抑

制剂的RIPA裂解液裂解细胞。提取总蛋白后,每份样品取10 μg总蛋白进行常规SDS-PAGE。结束后采用半干转印法(80 mA横流,电转印1.5 h)将PAGE胶上的蛋白电转印至PVDF膜上。使用5%脱脂奶粉(溶解于TBS-T缓冲液中)封闭PVDF膜,室温孵育1 h。向PVDF膜上滴加一抗工作液,4 ℃孵育过夜;次日向PVDF膜上滴加二抗工作液,室温孵育1 h。使用ECL化学发光底物对目的条带进行显影。抗体工作液稀释度:兔抗人HIF-1α(1∶2 000)、兔抗人VEGF-A(1∶2 000)、兔抗人GAPDH(1∶2 000)。实验独立重复3次。

1.8 转染

将CircPDS5B shRNA 5′-CAAAGACTAGGACCAATGATG-3′、shRNA NT 5′-TATAGCATAGCAGCAATCTAG-3′连接入慢病毒载体pCLenti-U6-shRNA-CMV-EGFP-WPRE中,分别构建Lenti-sh-CircPDS5B和Lenti-sh-NT慢病毒载体。将CircPDS5B-OE基因扩增产物连接入慢病毒载体pLenti-CMV-S-circRNA-WPRE中,构建Lenti-OE-CircPDS5B及Lenti-OE-Vector(仅有慢病毒载体)。CircPDS5B-OE基因扩增引物:CircPDS5B-OE正向引物5′-GGGAGAAGGCGATTGGATG-3′,反向引物5′-TAGCCTGCTGCCTTGCTGAT-3′。

不做转染处理的hPMEC细胞设为Control组。使用Lenti-sh-CircPDS5B(Lenti-sh-CircPDS5B组)、Lenti-sh-NT(Lenti-sh-NT组)、Lenti-OE-CircPDS5B(Lenti-OE-CircPDS5B组)和Lenti-OE-Vector(Lenti-OE-Vector组)慢病毒颗粒分别转染hPMEC。按照慢病毒颗粒感染的常规方法,加入无血清DMEM培养液稀释的对应分组的慢病毒颗粒,使其最终滴度为1.0×10^9 TU/mL。更换细胞培养液为无血清、无抗生素的DMEM培养液,加入上述稀释好的慢病毒颗粒,将细胞培养物置于37 ℃细胞孵育箱培养12 h。结束后,弃无血清细胞培养液,更换为含10%胎牛血清的完全DMEM培养液,继续培养细胞48 h后收集细胞样本,采用Western blot法检测靶蛋白。

1.9 统计学处理

定量数据以均数±标准差($\bar{x}\pm s$)表示。使用GraphPad Prism v8软件进行统计学分析。两组间差异比较采用t检验;多组间差异比较采用单因素方差分析和事后*Bonferroni*检验。$P<0.05$为差异具有统计学意义。

2 结果

2.1 早产儿胎盘组织CircRNAs表达谱微阵列分析结果

CircRNAs微阵列分析结果显示,微阵列芯片上具有14 236个人类CircRNAs探针,本研究检测到1 049个CircRNAs的表达。与Group 1相比,在Group 2~4中,前10位显著上调和前10位显著下调的CircRNAs见图1,其中CircPDS5B在Group 4中上调最显著($P<0.05$)。

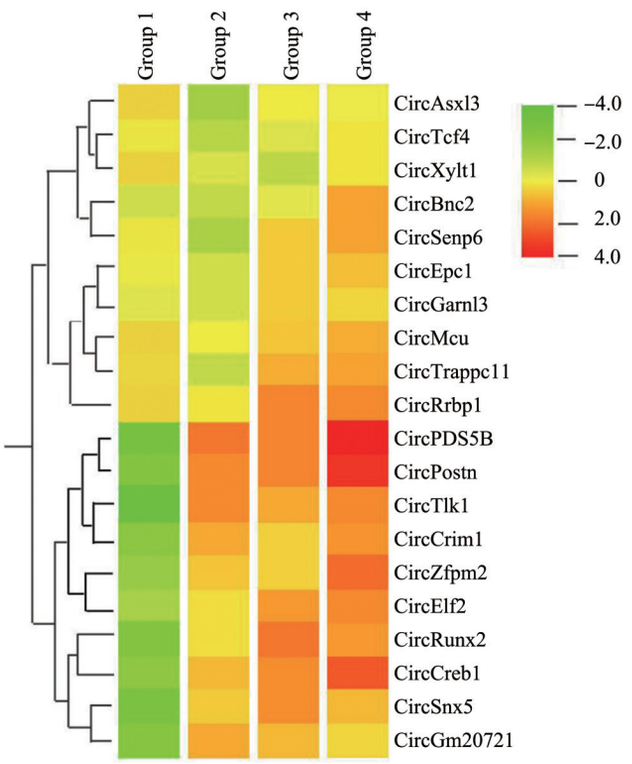


图1 前10位显著上调和前10位显著下调的CircRNAs热图

2.2 qPCR验证早产儿胎盘组织中显著差异表达的CircRNAs结果

与Group 1相比,在Group 2~4胎盘组织中显著上调的前10位CircRNAs,包括CircPDS5B、CircPostn、CircTlk1、CircCrim1、CircZfpm2、CircElf2、CircRunx2、CircCreb1、CircSnx5、CircGm20721;显著下调的前10位CircRNAs,包括CircRrbp1、CircTrappc11、CircMcu、CircGarnl3、CircEpc1、CircSenp6、CircBnc2、CircXylt1、CircTcf4、CircAsxl3。经qPCR法验证结果显示,CircPDS5B在早产儿胎盘组织中显著上调($P<0.05$),见表2、3。

2.3 验证CircPDS5B与hnRNPL及hnRNPL与HIF-1α mRNA的直接结合关系

Western blot实验结果显示,使用抗hnRNPL抗体下拉的hnRNPL能够检测到hnRNPL蛋白,10% Input能够检测到hnRNPL蛋白,而使用抗IgG抗体下拉,即IgG无法检测到hnRNPL蛋白,见图2。

RIP实验结果显示,与IgG组相比,hnRNPL组CircPDS5B和HIF-1α mRNA富集水平升高($P<0.05$),U6富集水平无明显差异($P>0.05$),见表4。

表2 各组胎盘组织中上调前10位CircRNAs的表达水平($\bar{x}\pm s$)

CircRNAs	Group 1	Group 2	Group 3	Group 4	F	P
CircPDS5B	1.00±0.18	7.73±1.12*	9.28±1.34*	13.77±1.67*	114.40	<0.001
CircPostn	1.00±0.13	5.56±1.16*	5.82±1.28*	5.95±1.11*	32.49	<0.001
CircTlk1	1.00±0.11	4.53±0.42*	6.57±0.61*	7.14±0.55*	214.30	<0.001
CircCrim1	1.00±0.07	4.38±0.72*	5.71±0.77*	10.63±1.16*	155.50	<0.001
CircZfpm2	1.00±0.27	3.34±0.88*	4.84±1.21*	5.43±1.18*	25.28	<0.001
CircElf2	1.00±0.06	3.22±1.08*	2.67±0.63*	4.35±1.37*	13.56	<0.001
CircRunx2	1.00±0.09	2.74±0.64*	2.88±0.42*	2.91±0.33*	29.19	<0.001
CircCreb1	1.00±0.12	2.33±0.18*	3.24±0.32*	3.46±0.25*	141.60	<0.001
CircSnx5	1.00±0.08	2.47±0.22*	2.57±0.18*	2.76±0.27*	98.11	<0.001
CircGm20721	1.00±0.14	1.87±0.16*	2.38±0.27*	2.45±0.21*	66.16	<0.001

*:与Group 1相比,P<0.05

表3 在各组胎盘组织中下调前10位CircRNAs的表达水平($\bar{x}\pm s$)

CircRNAs	Group 1	Group 2	Group 3	Group 4	F	P
CircRrbp1	1.00±0.22	0.16±0.06*	0.08±0.01*	0.03±0.02*	95.75	<0.001
CircTrappc11	1.00±0.12	0.37±0.02*	0.17±0.04*	0.07±0.01*	254.40	<0.001
CircMcu	1.00±0.07	0.73±0.04*	0.22±0.03*	0.08±0.01*	593.80	<0.001
CircGarnl3	1.00±0.14	0.81±0.04*	0.32±0.05*	0.24±0.03*	133.90	<0.001
CircEpc1	1.00±0.11	0.73±0.07*	0.82±0.03*	0.16±0.07*	138.60	<0.001
CircSenp6	1.00±0.07	0.92±0.10*	0.73±0.06*	0.47±0.07*	56.96	<0.001
CircBnc2	1.00±0.13	0.46±0.06*	0.63±0.12*	0.54±0.08*	33.10	<0.001
CircXylt1	1.00±0.12	0.64±0.06*	0.53±0.02*	0.17±0.04*	140.20	<0.001
CircTcf4	1.00±0.13	0.62±0.03*	0.22±0.04*	0.07±0.01*	214.90	<0.001
CircAsxl3	1.00±0.23	0.52±0.02*	0.37±0.07*	0.36±0.02*	37.03	<0.001

*:与Group 1相比,P<0.05

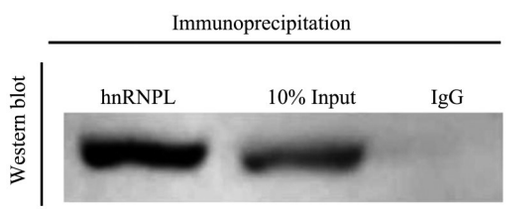


图2 RIP的免疫印迹检测结果图

表4 RIP实验证明hPMEC中CircPDS5B与hnRNPL及hnRNPL与HIF-1α mRNA的直接结合($\bar{x}\pm s$)

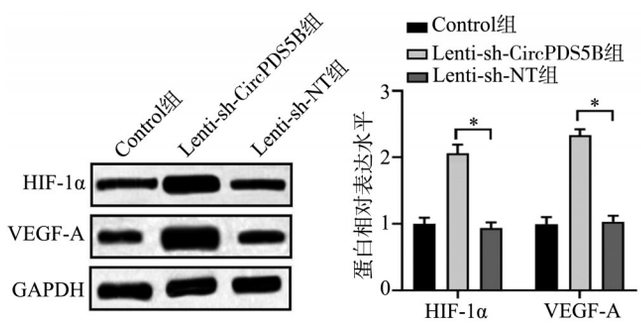
组别	U6	CircPDS5B	HIF-1α mRNA
IgG组	1.00±0.11	0.97±1.13	1.04±0.08
hnRNPL组	1.03±0.08	7.34±0.82	4.72±0.56
P	0.721 9	0.000 2	0.000 4

2.4 敲低CircPDS5B上调HIF-1α和VEGF-A的表达

Western blot结果显示,与Lenti-sh-NT组相比,Lenti-sh-CircPDS5B组细胞中HIF-1α和VEGF-A的表达水平升高(P<0.05);与Control组相比,Lenti-sh-NT组细胞中上述指标差异无统计学意义(P>0.05),见图3。

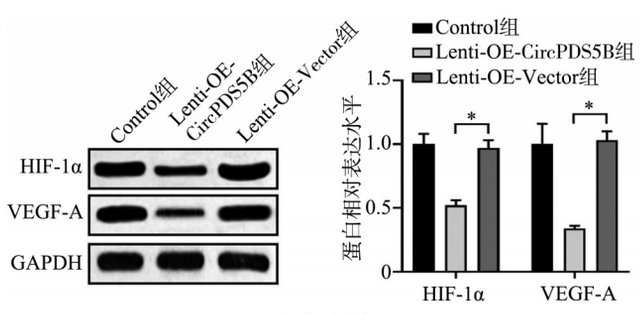
2.5 过表达CircPDS5B抑制HIF-1α和VEGF-A的表达

Western blot结果显示,与Lenti-OE-Vector组相比,Lenti-OE-CircPDS5B组细胞中HIF-1α和VEGF-A的表达水平降低(P<0.05);与Control组相比,Lenti-OE-Vector组细胞中上述指标差异无统计学意义(P>0.05),见图4。



*:P<0.05

图3 Western blot测定敲低CircPDS5B前后HIF-1α和VEGF-A的表达水平



*:P<0.05

图4 Western blot测定过表达CircPDS5B前后HIF-1α和VEGF-A的表达水平

3 讨论

目前关于CircRNAs表达谱差异与早产的病理生理机制的关系尚无研究进行系统、深入地探讨。本研

究发现,相对于足月儿胎盘组织中的CircRNAs,不同孕周早产儿胎盘组织中CircRNAs的表达变化显著,特别是前10位上调和前10位下调的CircRNAs,说明这些CircRNAs参与了早产的发病机制。已有报道显示CircPostn在胶质母细胞瘤中通过调节miR-219a-2-3p/STC1轴刺激VEGF-A分泌促进新生血管的形成[9]。CircTlk1通过miR-136-5p/Bcl2信号轴缓解氧-糖剥夺/再灌注诱导的人肾小管上皮细胞凋亡[10]。衰老过程中CircCrim1低表达通过miR-455-3p/Twist1/VEGFR2轴引起血管生成受损[11]。在阿尔茨海默病小鼠模型中,低氧预处理脂肪干细胞外泌体通过传递CircEpc1和改变小胶质细胞M1/M2极化、改善认知[12]。以上结果说明本研究检测出的显著上调的CircRNAs(CircPostn、CircTlk1、CircCrim1)和显著下调的CircRNAs(CircEpc1)均与低氧和血管发生机制相关。特别是CircPDS5B,通过与hnRNPL相互作用使VEGF-A失活,在缺血性卒中后损伤脑血管新生[13]。CircPDS5B水平降低通过microRNA-223-3p/NOTCH2轴促进缺血性卒中后血管生成[14]。且与健康对照组相比,中国人群急性缺血性卒中患者外周血中有3个CircRNAs表达显著上调,其中就包括CircPDS5B[15]。说明CircPDS5B能够通过抑制VEGF-A的表达,参与血管生成相关分子生物学过程。CircRNAs调节基因表达通过两种机制,一种是充当miRNA海绵体,调节靶基因mRNA的表达;另一种是通过RBPs稳定结合和隔离相关转录因子,调节下游靶基因mRNA的表达。本研究发现,CircPDS5B通过hnRNPL稳定结合并隔离了HIF-1α mRNA,使其无法与RNA聚合酶和核糖体结合并表达为HIF-1α蛋白。

目前研究表明,发生早产的机制可能包括子宫肌细胞的间隙性连接机制、内分泌调节机制、免疫机制和内皮功能障碍致血运构建不良机制等[16]。其中VEGF是胎盘血管发育的关键调节因子,其水平下调是血运构建不良机制致胎盘功能障碍的重要原因之一[17]。早产儿胎盘组织中VEGF的表达明显低于足月儿胎盘组织[18]。VEGF、血管生成素-1(angiopoietin-1,Ang-1)、血小板源性生长因子同二聚体A亚型(PDGF-AA)、成纤维细胞生长因子a和成纤维细胞生长因子b等促血管生成因子的水平在早产儿脐带血中显著降低[19]。感染疟疾导致的血管生成和炎症调节的关键因子失调与早产风险增加相关[20]。胎盘生长因子是VEGF家族成员之一,其均在血管生成中起着关键作用。研究表明,与对照组相比,早产病例母亲外周血浆中胎盘生长因子的水平显著下调[21]。子痫前期胎盘功能受损导致胎盘组织持续缺氧,HIF-1α/蛋白激酶B/磷脂酰肌醇3-激酶被持续激活,介导滋养层细胞损伤[22]。HIF-1α是一种缺氧诱导激活的转录因子,参与子痫前期胎盘血运构建异常的病理机制。子痫前期HIF-1α表达上调并介导长链非编码RNAs LncRNA-HEIPP,微小RNAs miR-126和miR-210,以及CircRNAs Circ_0090100、CircPCNXL2、CircPAPPA2等的表达上调,导致滋养层细胞侵袭不足和螺旋动脉重塑障碍[23-27]。胎盘缺氧上调了HIF-1α/Notch1/ETBR的表达,促进了滋养细胞的侵袭和血管生成[28]。在先兆早产和无并发症早产产妇血清中,HIF-1α和白细胞介素-6水平均显著高于足月产妇[29]。在小鼠产前缺氧模型中,妊娠后期短暂的产前缺氧增加了胎儿大脑中HIF-1α的蛋白水平[30]。抑制HIF-1α介导的Toll样受体4激活可减少胎盘微血管内皮细胞凋亡并促进血管生成[31]。因此,胎盘缺氧会刺激HIF-1α表达上调。HIF-1α是转录VEGF-A的重要转录因子。低氧/缺氧诱导的HIF-1α表达上调,随后通过转录VEGF增加组织内供氧[32-33]。本研究发现,敲低CircPDS5B降低了hnRNPL与HIF-1α mRNA的稳定结合和对其的隔离作用,释放了更多的游离HIF-1α mRNA,上调HIF-1α的表达,增加VEGF-A的转录;过表达CircPDS5B增加了hnRNPL与HIF-1α mRNA的稳定结合和隔离作用,减少了游离HIF-1α mRNA,下调HIF-1α的表达,抑制VEGF-A的转录。因此可以推断,低氧诱导CircPDS5B表达上调,抑制了HIF-1α的表达,下调了VEGF-A的转录,可能参与介导阻遏血管发生。后续研究需要在细胞水平上建立"低氧诱导的细胞模型",进一步探讨低氧是否是调节CircPDS5B表达的关键作用因子,以及具体的调节机制。

综上,早产儿胎盘组织中CircPDS5B的水平显著上调,并通过hnRNPL稳定结合和隔离HIF-1α mRNA抑制VEGF-A的转录参与胎儿早产的病理生理机制。

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