复发性流产患者子宫蜕膜基因表达谱

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摘要 从基因组角度分析复发性流产(recurrent spontaneous abortion, RSA)患者子宫蜕膜异常表达基因, 为寻找不明原因RSA可能的发生机制及筛选特异性标志分子提供实验依据。分离RSA患者和正常早孕妇女的子宫蜕膜, 提取RNA标记后用于表达谱芯片杂交, 生物信息学分析芯片数据, RT-PCR验证芯片结果。结果显示, 与正常对照相比较, RSA患者蜕膜中共发现差异表达基因1 656个, 其中上调基因1 184个, 下调基因472个。基因功能分类显示差异表达基因涉及多个生物学过程和功能, 包括代谢、细胞与细胞间作用、生殖、发育、外来刺激应激、细胞增殖、补体激活等。信号通路分析显示, 8个信号通路可能参与了RSA的发生, 包括FoxO家族(FoxO family signaling)和Akt介导Class I PI3K(Class I PI3K signaling events mediated by Akt)等信号通路。半定量PCR验证表达谱芯片中RSA患者蜕膜异常表达基因, 与正常对照相比较, *DKK1*、*GADD45A*和*GNLY*基因表达显著上调, 而*DLK1*的表达则显著下调, 结果与基因芯片的结果有较好的一致性。RSA患者子宫蜕膜基因表达谱发生显著改变, 这与RSA的发生密切相关。

关键词 复发性流产; 子宫蜕膜; 基因表达谱

Gene Expression Profile in Decidua of Patients with Recurrent Spontaneous Abortion

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Abstract To provide experimental basis for discovering possible mechanism of RSA and specific markers screening, the abnormal gene expression in uterine decidua of patients with recurrent spontaneous abortion was analyzed by genome resorts. Uterine decidua of patients with RSA and normal early pregnant were collected and RNA was extracted for microarray hybridization and the microarray data was analyzed by bioinformatics analysis. In addition, we validated the chip results with RT-PCR. The results showed that compared with control group, 1 656 differentially expressed genes were found in patient's decidua with RSA, among which, 1 184 up-regulated genes while 472 down-regulated genes were found. Differentially expressed genes involved in multiple biological processes and functions, including metabolism, cell-cell interaction, reproduction, development, external stimulus such as stress, cell proliferation and complement. Signal pathway analysis showed 8 signaling pathways may involved in the progression of RSA, including FoxO family and Akt-mediated Class-I PI3K signaling pathways. Abnormal expression

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in microarray gene of patient's decidua with RSA was verificated by semi-quantitative PCR. Compared with control group, the expression of DKK1, GADD45A and GNLY genes were increased significantly while the DLK1 expression was significantly reduced, which indicated consistency with the results of gene chip. Gene expression profiling significantly changed in patient's decidua with RSA is closely related with RSA.

Key words recurrent spontaneous abortion (RSA); decidua; gene expression profile

复发性流产(recurrent spontaneous abortion, RSA)是指女性有2次或2次以上在早孕期自然流产史, RSA患者约占育龄妇女的3%, 是困扰育龄期妇女的常见疾病之一[1]。RSA的病因主要源自两个方面, 一是胚胎的发育异常; 二是母体异常。母体原因致RSA的主要有: (1)母体自身染色体异常: 常见的染色体异常为平衡易位、罗伯逊易位等; (2)内分泌异常: 包括黄体功能不全、多囊卵巢综合症、高泌乳素血症、甲状腺疾病、糖尿病等; (3)生殖道解剖结构异常: 包括子宫畸形、asherman综合症、宫颈机能不全、子宫肌瘤等; (4)生殖道感染: 沙眼衣原体、解脲支原病毒感染等; (5)免疫功能异常: 抗磷脂抗体综合症和免疫调节和抑制细胞失衡等[2]。但是, 现在仍大约有40%的RSA患者流产原因尚未阐明[3], 其中可能源自母体的未知异常又占据较大比例。

胚胎植入过程中, 胚胎植入前子宫容受态形成和胚胎植入后子宫内膜蜕膜化都与胚胎植入和妊娠维持密切相关[4], 其中的任一因素异常均可致母胎识别失败导致早期流产[5-6]。近年来, 对原因不明复发生性流产患者子宫内膜及其蜕膜化的研究逐渐成为热点, 有研究表明RSA与子宫内膜功能异常密切相关[7]。子宫内膜蜕膜化是子宫内膜基质细胞受到蜕膜化诱导因子的刺激而增殖和再分化, 腺体和螺旋小动脉发育以及自然杀伤细胞的聚集, 为胚胎提供营养和调节滋养层减少局部免疫反应, 它是胚胎植入母体必不可少的条件[8], 缺乏蜕膜化或蜕膜化不足的子宫内膜将导致胚胎植入失败[9]。在蜕膜化过程中自然杀伤细胞(NK细胞)功能异常[10]、血管生成过程异常[11]、细胞因子如白血病抑制因子(LIF)分泌异常[12-13]、蜕膜细胞凋亡增加、细胞增殖下降[14]、蜕膜化相关的基因表达异常等[15]均可导致妊娠失败。

近年来, 从全基因组角度研究RSA发生的病因学取得了更大进展, Othman等[16]利用基因芯片研究了RSA患者分泌期子宫内膜的基因表达谱, 显示*FGF9*、*ITGB3*、*CSF1*和*MMP19*等基因表达异常。Lee等[17]利用基因芯片分析植入窗口期RSA患者子宫内膜, 发现了29个与着床相关的基因表达异常, 但是目前利用RSA患者蜕膜进行流产相关病因的研究仍未见报道。本研究收集了RSA患者和正常早孕期终止妊娠的女性的子宫蜕膜, 筛查了与RSA发生相关的基因群, 分析差异表达基因的功能。其结果对于寻找不明原因RSA可能的发生机制及筛选特异性标志分子具有重要意义。

1 材料与方法

1.1 材料

病因不明的反复自然流产(RSA)患者(n=5)和正常对照早孕妇女(n=5)子宫蜕膜均收集自重庆医科大学附属第一医院。病因不明的反复自然流产病例筛查方法: 依据病史、体检、妇检、B超检查、性激素测定[雌二醇(E2)、孕酮(P)、催乳素(PRL)]、自身抗体检测[可提取的核抗原抗体(ENA)、抗核抗体(ANA)、抗心磷脂抗体(ACL)]、男方精液分析、流产胚胎刮出物滋养叶细胞染色体核型分析, 以排除遗传、解剖、内分泌及自身免疫异常。RSA观察组: 病史与B超检查示胚胎停止发育, 包括胚囊比孕周小或未见胚芽或未见胎儿原始心血管搏动, 平均年龄为(32.2±1.6)岁, 孕周为(9.7±2.3)周。正常对照组: 正常妊娠, 要求行人工流产, 无急、慢性疾病史, 同时B超检查显示胚胎发育正常, 平均年龄为(32.1±1.5)岁孕, 孕周为(8.1±2.2)周。所选病例均已排除生殖道解剖结构异常、生殖道感染, 生殖内分泌检查正常, 自身抗体检查ENA、ANA、ACL检查均阴性, 男方精液分析和流产胚胎染色体检查均正常。标本其他具体资料见表1。研究得到重庆医科大学健康与伦理委员会批准, 所有临床病理及正常对照材料的收集均得到病人知情同意。

1.2 方法

1.2.1 RNA抽提与Affymetrix基因芯片杂交 总RNA抽提参照RNeasy kit(Invitrogen)说明书进行, 并用Reeasy kit(Qiagen)纯化, 并经分光光度计($D_{280/260}$)和2.0%琼脂糖凝胶电泳检测总RNA浓度和片段完整

表1 入选标本临床资料

Table 1 Demographic data of the 10 subjects

组别 Group	编号 Serial number	年龄(岁) Age	孕周(周) Gestational age(weeks)	自然流产次数 Frequency of spontaneous abortion	孕囊大小(mm) The sizes of fertilized egg(mm)
RSA	1	31	7.4	3	12
	2	30.8	8.5	3	19
	3	32.2	9.6	3	27
	4	32.2	11	3	38
	5	33.8	12	4	52
Control	1	32	6	0	12
	2	30.6	7	0	19
	3	32.2	8.2	0	28
	4	33.6	9	0	36
	5	32.4	10.3	0	50

表2 RT-PCR引物

Table 2 Primers for RT-PCR

基因 Gene	正向引物 Primer(forward)	反向引物 Primer(reverse)	产物大小(bp) Size(bp)	退火温度(°C) Tm(°C)
DLK1	5′-CTG AAG GTG TCC ATG AAA GAG-3′	3′-GCT GAA GGT GGT CAT GTC GAT-5′	273	60
GADD45A	5′-GAG AGC AGA AGA CCG AAA GGA-3′	3′-CAC AAC ACC ACG TAT CGG G-5′	200	55
GNLY	5′-GGC CGT GAC TAC AGG ACC TGT C-3′	3′-CCT GAG GTC CTC ACA GAT CTG-5′	222	60
DKK1	5′-ATT CCA ACG CTA TCA AGA ACC-3′	3′-CCA AGG TGC TAT GAT CAT TAC C-5′	383	55
ACTB	5′-GTC GGC CGC TCT AGG CAC CAA-3′	3′-CTC TTT GAT GTC ACG CAC GAT TTC-5′	540	55

性。5名RSA患者子宫蜕膜细胞总RNA混合后作为病例组基因池, 5名正常女性子宫蜕膜细胞总RNA混合后作为对照组基因池。纯化后的总RNA采用Super Script Double-Stranded cDNA Synthesis Kit (Invitrogen)试剂盒行逆转录并合成cDNA链, 再由Enzo BioArray High Yield RNA T7 Transcript Labeling Kits(Enzo)体外合成互补的cRNA。纯化的cRNA片段化为大小为50~200 bp大小的片段之后, 将每组含有10 μg cRNA的200 mL杂交混合液与HG U133Plus 2.0表达谱芯片进行杂交, 洗涤之后用streptavidin phycoerythrin染色。第二次洗涤后与生物素标记anti-streptavidin phycoerythrin孵育。streptavidin phycoerythrin染色后, GeneChip Scanner 3000(Affymetrix)扫描荧光信号GeneChip Operating software (GCOS)提取数据采用。

1.2.2 数据归一化, 质量控制分析及探针筛选 将GCOS产生的RSA组和对照组探针杂交信号数据导入GeneSpring GX 9.0.5 软件(Agilent)。含有探针信号的CEL格式数据经Robust Multichip Average(RMA)检测和基线数据中位数换转后进行质量控制分析, (1)内标质量控制: 选择GAPDH为内标分析3′/5′比值, 比值小于3.0则RNA质量达到要求; (2)杂交质量控制: 分析不同浓度(1.5, 5, 25, 100 ng/μL)生物素标记cRNA混合物(bioB, bioC, bioD和cre)与不同样本杂交信号之间的差异; (3)主成分分析(PCA): 采用图形工具识别所有基因在不同样本中的表达趋势。最后, 根据探针杂交信号强度值, 并通过设定表达信号百分位数限定值(低位20.0%, 高位100.0%), 在95%可信限下去除无关基因探针, 完成探针过滤。

1.2.3 生物信息学分析 $\log_2$转化差异信号比值数据后进行基因差异表达数据分析。选择差异表达基因的标准为表达差异值≥3.0(表达上调≥3.0; 表达下调≤–3.0)。采用欧几里德距离法(Euclidean distance)进行全部差异基因表达模式相似性的层次化聚类(hierarchical clustering)。差异表达基因功能分类采用Genespring中Gene Ontology(GO)功能元件分析, 信号通路分析Find Similar Pathway tool元件进行。

1.2.4 半定量RT-PCR 芯片杂交试验中分装的纯化后总RNA经PrimeScriptTM RT reagent kit(TaKaRa, 大连)逆转录为cDNA。半定量RT-PCR在Master cycler

PCR Cycler PCR仪(Eppendorf)上完成。4个基因(表2)用于验证芯片质量。PCR循环条件为94 °C 4 min; 94 °C 30 s, T_m 30 s, 72 °C 30 s, 25-35个循环。不同基因PCR扩增循环数如下: *DLK1*和*GADD45A*为30个循环; *GNLY*为32个循环; *DKK1*为28个循环; *ACTB* 26个循环。PCR产物经2%凝胶电泳后, *ACTB*作为内参照, Quality one软件分析条带灰度值。

2 结果

2.1 样本和芯片杂交质量控制

数据归一化后, 进行样本质量控制评价。总RNA样本质量经GAPDH为内标分析3′/5′比值, 结果显示, GAPDH和其他基因的3′/5′比值均小于3.0(表3), 显示RNA质量适用于进一步的标记并用于芯片杂交。

芯片杂交质量控制中, bioB信号的阳性信号表示两组芯片能够检测到的杂交信号最低值, 其他对照如bioC、bioD和cre等杂交信号增强显示芯片杂交反应、随后的洗涤和染色步骤均无问题(图1)。

主成分分析(PCA)所有基因在不同样本中的表达趋势显示, PCA点图之间距离较大, 显示RSA组基因表达与对照组基因表达差异明显(图2)。

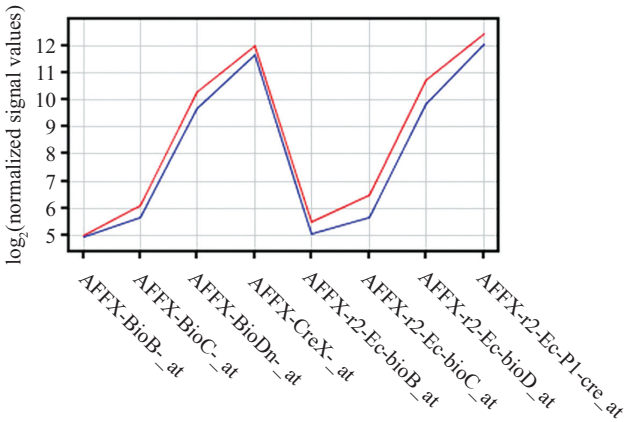
2.2 差异表达基因分析

数据归一化后, 根据基因差异表达信号百分位数限定值(95%可信限), 获得45 205个转录本(蓝色), 差异表达基因9 470个(绿色)。表达差异≥3或≤–3倍的基因1 656个(红色, 图3), 包括上调基因1 184个, 下调基因472个。

表3 内标对RNA样本质量控制

Table 3 RNA sample quality was assessed by internal controls

探针ID Probe ID	对照组3′/5′比例 3′/5′ ratio in control	RSA组3′/5′比例 3′/5′ ratio in RSA
X00351-at	1.496 273	1.405 663
M33197-at	1.444 118	1.181 056
M97935-at	1.473 705	2.205 426
M10098-at	0.465 722	0.433 269
M27830-at	0.120 293	0.560 982

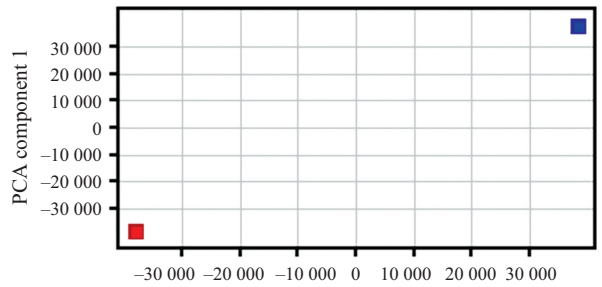


图中X轴代表对照组, Y轴代表标准化信号对数值, 红色为对照组, 蓝色为RSA组。

The X-axis in this graph represents the controls and the Y-axis, represents the log of the normalized signal values. Red: control; Blue: RSA.

图1 芯片杂交质量控制

Fig.1 Profile hybridization quality control

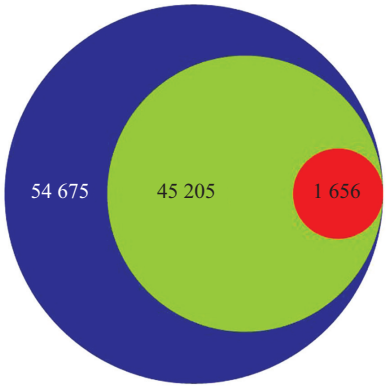


X和Y轴显示任意单位独立组之间表达值差异, 红色代表对照组, 蓝色代表RSA组。

X and Y-axis shows distinction between the expression values of the individual groups in arbitrary units. Red: control; Blue: RSA.

图2 PCA图

Fig.2 The PCA plot



一共有54 675个探针集合(集合1, 蓝色)用来杂交, 45 205个工作基因列表(集合2, 绿色)在滤过时产生, 1 656个基因经鉴定为差异表达基因(集合3, 差异倍数≥3.0, 红色)。

A total of 54 675 probe sets (list 1, blue) were used in the hybridization. When filtered, working gene list (list 2, green) consisting of 45 205 was produced. 1 656 genes were identified as differentially expressed (list 3, fold change≥3.0, red).

图3 Venn图

Fig.3 Venn diagrams

表4 RSA组与正常对照组蜕膜表达差异最大的40个转录本

Table 4 The 40 most abundant transcripts present in RSA and normal control(RSA *vs* control)

基因名称(基因符号) Gene title(gene symbol)	基因号 Unigene(avadis)	差异倍数 Fold change
SPARC-like 1(mast9 hevin)(*SPARCL1*)	Hs.62886	+139.67
Granulysin(*GNLY*)	Hs.105806	+129.86
Insulin-like growth factor binding protein 7(*IGFBP7*)	Hs.479808	+86.74
Insulin-like growth factor binding protein 1(*IGFBP1*)	Hs.642938	+83.41
Solute carrier family 1(*SLC1*)	Hs.444915	+82.77
Growth arrest and DNA-damage-inducible alpha(*GADD45A*)	Hs.80409	+80.19
Dickkopf homolog 1(*Xenopus laevis*)(DKK1)	Hs.40499	+80.00
Granzyme A(*GZMA*)	Hs.90708	+70.16
Family with sequence similarity 148 member A(*C2CD4A*)	Hs.202656	+66.43
Insulin-like growth factor binding protein 2(*IGFBP2*)	Hs.438102	+54.40
Transmembrane protein 45A(*TMEM45A*)	Hs.658956	+51.86
Iroquois homeobox 3(*IRX3*)	Hs.499205	+49.72
Complement component 1 subcomponent(*C1R*)	Hs.524224	+49.67
Similar to Complement C3 precursor(*C3*)	Hs.529053	+47.98
Dynein light chain Tctex-type 3(*DYNLT3*)	Hs.446392	+46.27
DEP domain containing 6(*DEPDC6*)	Hs.112981	+45.94
Integrin beta 8(*ITGB8*)	Hs.592171	+44.97
Pantigen family member 4(*PAGE4*)	Hs.441038	−152.14
Delta-like 1 homolog (*Drosophila*)(*DLK1*)	Hs.533717	−138.66
Pleckstrin homology-like domain family A member 2(*PHLDA2*)	Hs.154036	−124.09
Hemoglobin gamma G(*HBG2*)	Hs.302145	−85.26
Mesoderm specific transcript homolog(mouse)(*MEST*)	Hs.270978	−82.75
Proteoglycan 2 bone marrow(*PRG2*)	Hs.512633	−80.89
Tissue factor pathway inhibitor 2(*TFPI2*)	Hs.438231	−77.57
Growth hormone 1(*GH1*)	Hs.655229	−72.34
Chorionic somatomammotropin hormone 1(*CSH1*)	Hs.654390	−70.15
Hemoglobin epsilon 1(*HBB*)	Hs.655195	−64.95
Paternally expressed 3(*PEG3*)	Hs.201776	−64.82
HtrA serine peptidase 4(*HTRA1*)	Hs.661014	−59.85
Pregnancy specific beta-1-glycoprotein 9(*PSG9*)	Hs.502092	−58.75
Hemoglobin zeta(*HBZ*)	Hs.585357	−55.68
Paternally expressed 10(*PEG10*)	Hs.147492	−54.20
Pregnancy specific beta-1-glycoprotein 6(*PSG6*)	Hs.654414	−53.57
S100 calcium binding protein P(*S100P*)	Hs.2962	−53.41
Leptin(obesity homolog mouse)(*LSL*)	Hs.194236	−52.52
Pregnancy specific beta-1-glycoprotein 2(*PSG1*)	Hs.709192	−51.14
KiSS-1 metastasis-suppressor(*KISS1*)	Hs.95008	−49.62
GATA binding protein 3(*GATA3*)	Hs.524134	−49.13
Hydroxy-delta-5-steroid dehydrogenase 3 beta- and steroid Delta-isomerase 1(*HSD3B2*)	Hs.364941	−48.94
Chorionic gonadotropinbeta polypeptide(*CGB*)	Hs.172944	−46.97

“+”显示表达上调, “−”显示表达下调。

“+” means up-regulated expression, “−” means down-regulated expression.

表5 RSA患者差异表达基因功能分类分析

Table 5 Gene ontology(GO) analysis of differentially expressed genes involved in RSA

GO登录号 GO accession	GO项目 GO term	*P*值 *P* value	入选数 Count in selection	入选数百分比(%) Count in selection(%)
GO:0008152	Metabolic process	0.10	184	25.73
GO:0009987	Cellular process	1.56E-03	162	22.66
GO:0022414	Reproductive process	9.37E-05	18	2.52
GO:0032501	Multicellular organismal process	4.0 E-03	123	17.2
GO:0032502	Developmental process	4.94E-05	12	1.68
GO:0050896	Response to stimulus	1.15E-06	132	18.46
GO:0051704	Multi-organism process	1.90E-07	18	2.52
GO:0065007	Biological regulation	1.43E-04	66	9.23

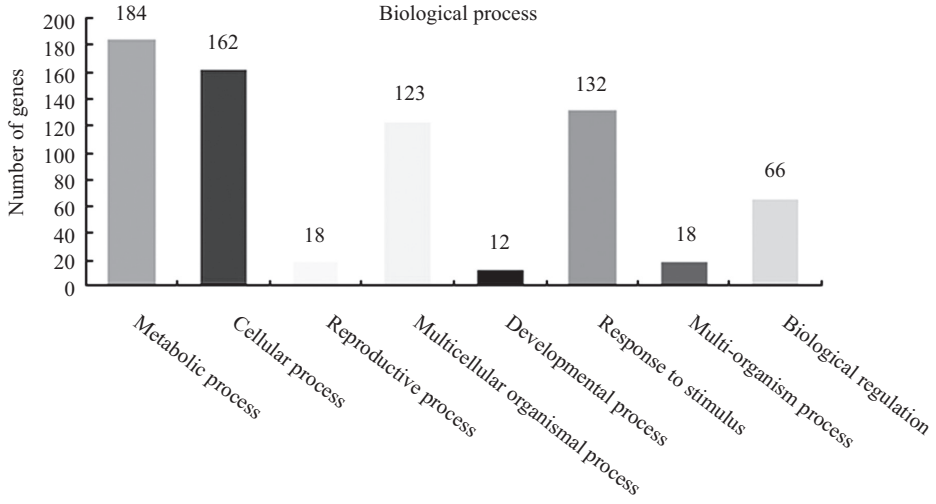


图4 1 656个RSA相关的基因本体表达谱

Fig.4 Gene ontology profile of the 1 656 RSA related genes

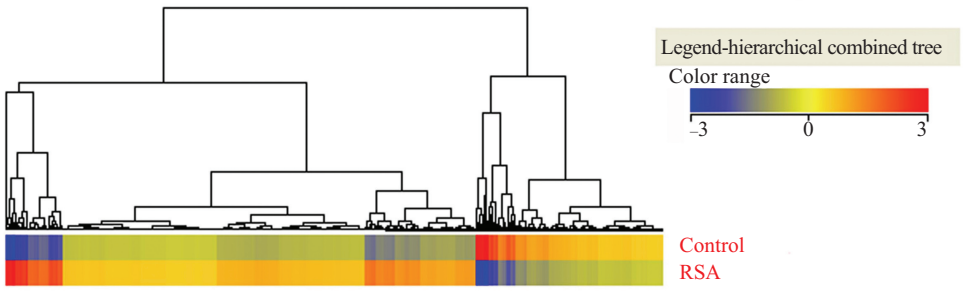
表4显示了RSA患者蜕膜中表达差异最大的40个基因, 其中部分已经已被证实与RSA发生相关, 如粒溶素基因(GNLY, +129倍), 瘦素(Leptin, –52.52倍), 胰岛素样生成因子结合蛋白3(IGFBP1, –5.4倍)和晶体状蛋白(CRYAB, +4.4倍)等。我们在RSA患者中发现了一些以前未报到过的差异表达基因, 这可能与RSA发生相关, 如显著下调表达的*PAGE4*、*PEG3*、*PEG10*等以及显著上调表达的*SPARCL1*、*IGFBP7*、*PSG6*、*PSG9*等。

2.3 GO分析

GO注释显示差异表达的1 656个基因与RSA发生相关, 涉及多个生物学过程和功能(表5和图4), 其中184个属于代谢过程, 162属于细胞与细胞间作用过程, 18个属生殖过程, 12个与发育过程相关, 132个与外来刺激应激相关。

2.4 RSA差异表达基因功能聚类分析

对1 656个差异表达基因聚类分析显示, RSA患者和正常对照之间基因表达存在较大差异(图5), 在RSA中表达上调的聚类(红色)有细胞增殖相关基因(*IGFBP7*、*IGFBP6*、*GAS1*、*PPAP2A*、*IGFBP5*、*TOB1*), 补体激活相关基因(*CD55*、*C4A*、*CLU*、*C1R*、*SERPING1*、*C1S*、*CFD*), 蛋白质水解相关基因(*GZMA*、*DNER*、*CPXM1*、*CAPN2*、*CFD*、*SRGN*), 发育过程负调控相关基因(*CRYAB*、*CLU*、*FOXO1*、*ID4*、*PRNP*、*SRGN*、*TOB1*); 氨基酸代谢过程相关基因(*KLRC2*、*IVNS1ABP*、*CFD*、*GNLY*、*MAOA*、*HLA-DPA1*); RSA中表达下调的基因聚类(蓝色)有调控生殖过程相关基因(*CSH1*、*PSG2*、*PSG3*、*PSG1*、*HMGA1*、*PSG9*、*ADM*、*PSG7*、*PSG6*、*PSG5*、*PSG4*、*CRH*和*LHB*), 激素合成过程相关基因(*HSD3B1*、*ADM*、*HSD17B1*、

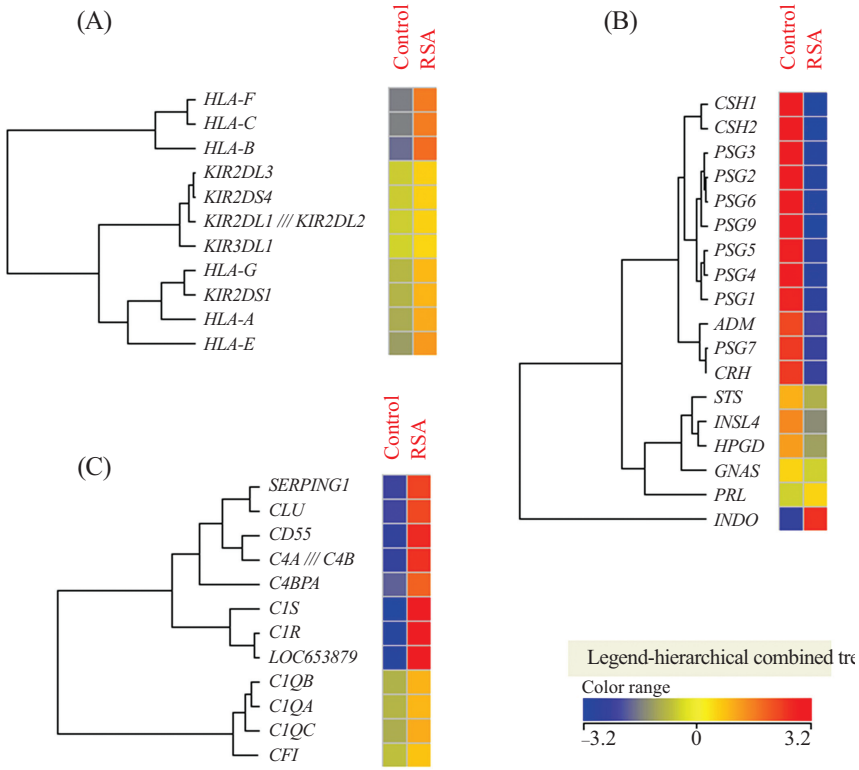


欧几里德距离法测量和≤–3.0到≥3.0色泽范围检测1 656个差异表达探针集合的标准化密度值对数, 红色代表基因表达上调, 黄色代表适中, 蓝色代表下调。

The normalized log intensity values for all 1656 differentially expressed probe sets were measured by Euclidean distance metric and colored on a range of ≤–3.0 to≥3.0. Red denotes up-regulated, yellow denotes intermediate, and blue denotes down-regulated expression levels.

图5 集簇差异表达基因热图

Fig.5 The heatmap of Clustered differentially expressed genes



A: MHC经典受体I活力; B: 妊娠相关; C: 补体激活。

A: MHC class I receptor activity; B: pregnancy related; C: complement activation.

图6 正常对照组和RSA患者组共表达基因中所选的差异表达基因

Fig.6 Selected clusters of differentially expressed genes that are coexpressed in decidua from control and RSA patients

CRH), 氧传输过程相关基因(*HBZ*、*HBG1*、*HBG2*和*HBE*), 揭示了RSA患者蜕膜基因表达与正常相比发生了较大的改变。

我们将可能与RSA发生密切相关的的基因聚类进行了突出显示(图6), 11个MHC class I受体激活基因(*HLA-G*、*HLA-A*、*HLA-E*、*HLA-F*、*HLA-C*、*HLA-B*、*KIR2DL3*、*KIR2DS4*、*KIR2DS1*、*KIR2DL1*和*KIR2DL2*)和13个补体基因(*SERPING1*、*CD55*、*C1QB*、*CFD*、*C4BPA*、*C4A*/*C4B*、*C1S*、*CLU*、*C1R*、*LOC653879*、*C1QA*、*C1QC*和*CFI*)的表达均显著上调; 与此相反与受孕或妊娠维持的18个基因中, 除了*PRL*和*INDO*外, 其余16个基因(*PSG1*、*PSG2*、*PSG3*、*PSG4*、*PSG5*、*PSG6*、*PSG7*、*PSG9*、*CSH1*、*CSH2*、*CRH*、*STS*、*INSL4*、*HPGD*和*GNAS*)的表达均显著下调。

表6 RSA涉及的信号通路

Table 6 Signal pathways involved in RSA

信号通路 Pathway	结点数 Number of nodes	本体数 Number of entities	匹配本体数 Number of matching entities	*P*值 *P* value
Signaling events medaited by HDAC Class I	178	79	2	0.040
IL6-mediated signaling events	56	29	2	0.006*
Angiopoietin receptor Tie2-mediated signaling	73	38	1	0.009*
Signaling events mediated by HDAC Class III	44	23	1	0.004*
IL2-mediated signaling events	164	75	3	0.041
FoxO family signaling	48	32	5	0.013
Class I PI3K signaling events mediated by Akt	148	66	4	0.034
Inositol phosphate metabolism	96	70	2	0.022

*P<0.01.

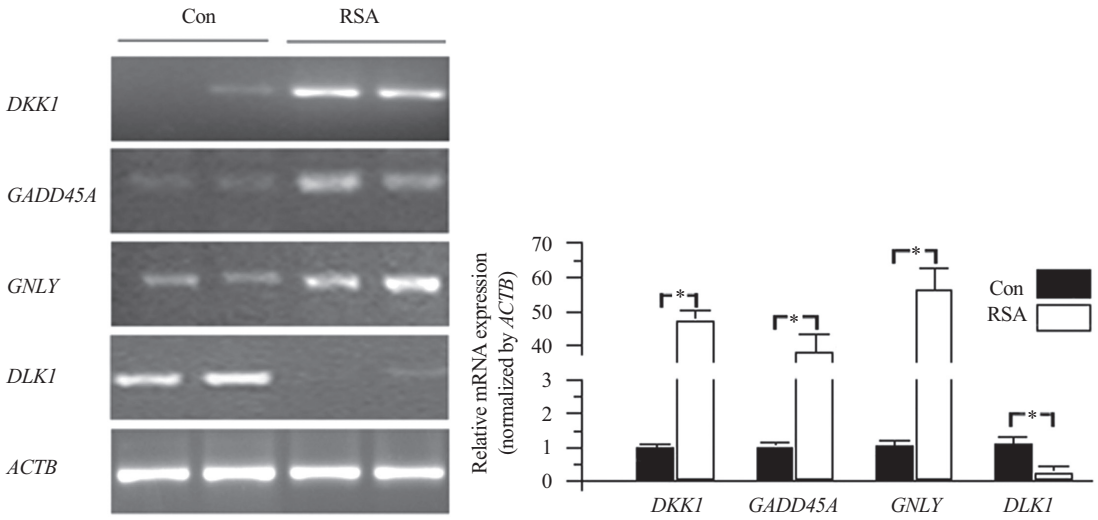


图7 半定量PCR检测RSA患者蜕膜中差异表达基因

Fig.7 Semi-quantitative RT-PCR validating of differentially expressed genes in RSA

2.5 细胞信号通路分析

信号通路分析显示, 8个信号通路可能参与了RSA的发生(表6), 其中FoxO家族信号通路(FoxO family signaling)和Akt介导Class I PI3K信号通路(Class I PI3K signaling events mediated by Akt)与预先导入的已知BioPAX 信号通路匹配的基因最多。FoxO家族信号通路中包括*CDK2*、*BCL6*、*FOXO1*、*FOXO3*和*FOXO4*基因, 这些基因在RSA患者中大多数呈上调表达。Akt介导Class I PI3K信号通路包括*CITED2*、*YWHAZ*、*FOXO1*和*FOXO3*。

2.6 半定量RT-PCR

半定量PCR验证表达谱芯片中RSA患者蜕膜异常表达基因, 结果显示与正常对照相比较, *DKK1*、*GADD45A*和*GNLY*基因表达显著上调, 而*DLK1*的表达则显著下调(图7)。这与基因芯片的结果有较好的一致性。

3 讨论

近年来的生物技术, 如胚胎选择技术的进步, 以及胚胎培养过程的完善, 极大地推动了生殖医学的快速发展。然而部分生殖医学领域的发展仍不显著, 目前, 仍有较高比例的育龄期女性遭受着RSA的困扰[1]。针对RSA的研究主要包括遗传和免疫因素异常、卵巢和子宫内膜功能异常等密切相关基因的表达等。利用基因芯片等全基因组检测技术已发现了更多的可能与RSA相关的异常表达基因。目前已被证实与RSA相关、源自母体的因素包括遗传、免疫因素异常[18]、解剖学结构异常[19]、病毒感染[20]、卵巢功能异常[21-23]、子宫内膜病变[4]、调控子宫生

理性周期变化基因表达异常[4]、细胞因子分泌异常[12-13]等。

本研究利用基因芯片技术检测了RSA患者蜕膜细胞异常表达基因, 共检测到1 656个差异表达基因, 并采用RT-PCR进一步验证了芯片数据的可靠性, 这揭示了RSA患者蜕膜基因的表达与正常相比发生了较大的改变。这些基因的功能涉及细胞增殖相关、补体激活、蛋白质水解、发育过程负调控、氨基酸代谢、生殖调控、激素合成、氧传输等。其中部分差异表达基因已被其他研究者证实与RSA的发生相关, 如*GNLY*的上调表达可诱导绒毛外滋养细胞的过度凋亡, 从而降低其侵入子宫蜕膜的能力, 引起RSA[24]; 瘦素的血清浓度在RSA患者中显著低于正常娩出胎儿的孕妇; 胰岛素样生成因子结合蛋白1(IGFBP1, –5.4倍)是子宫蜕膜化过程中的关键基因, IGFBP1表达降低可导致妊娠维持失败[25]; 晶体状蛋白(CRYAB, ＋4.4倍)可促进滋养细胞的生长发育和子宫内膜的蜕膜化, RSA患者蜕膜中CRYAB的表达显著高于对照组, 提示其表达异常与RSA发生相关[26]。我们在RSA患者中发现了一些以前未报到过的差异表达基因, 如显著下调表达的*PAGE4*、*PEG3*、*PEG10*等以及显著上调表达的SPARCL1、IGFBP7、PSG6、PSG9等。其中, *IGFBP7*受到我们的关注, 在多种肿瘤中, *IGFBP7*作为抑癌基因发挥作用, 有文献报道, IGFBP7在绒毛膜外滋养细胞中低表达而合体滋养细胞中高表达, 其机制可能是雌激素通过ER和TGF-β刺激IGFBP7表达升高, 从而抑制细胞的增殖和侵袭能力[27]。而本研究发现, *IGFBP7*在RSA患者蜕膜中表达显著上调, 推测可能是IGFBP7表达增高抑制了蜕膜细胞增殖, 使子宫内膜蜕膜化失败而导致流产, 通过哪种途径影响仍需进一步研究。

RSA的发生通常不是某一种或几种特定的因素造成的, 往往由一类或几类基因和其他因素共同参与。MHC class I受体激活基因母胎界面免疫调控。Flores等[28]报道了KIR receptors和HLA-C在RSA患者NK细胞中表达显著上调。我们的研究发现, MHC class I受体激活基因中有11个在RSA中的表达均显著上调, 进一步揭示了该基因家族在RSA发生中的作用。补体激活途径是介导母胎免疫的另一个重要系统, 补体激活系统调控异常将不利于胎儿在宫内的发育并致小鼠流产或子痫前期, 亦与病因不明的RSA发生相关[16,29]。此外, 精确调控的补体激活还参与了螺旋动脉的生成[30], 这与正常妊娠的维持关系密切。Young等[31]还观察到CD55在RSA患者分泌期子宫表达显著上调。本研究中, 我们在RSA中亦发现了13个补体激活显著上调表达的基因, 这均提示, 补体激活途径的异常可能是RSA发生的重要原因之一。

RSA的发生通常还涉及细胞间信号传导。Taylor等[32]发现, JAK/STAT信号通路关键分子JAK3、STAT5和NF-kappaB的表达均显著上调。本研究显示, 8个信号通路参与了RSA的发生, 但是只有两个信号通路中涉及的RSA相关的差异表达基因较多, FoxO家族信号通路(5个基因)亦参与了RSA的发生和Akt介导的Class I PI3K信号通路(4个基因)。这两个信号通路参与了多个生理过程[33-34], 但是在RSA中的作用仍需要进一步研究。

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