

# *ABCA1*对滋养细胞基因表达谱的影响

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**摘要** 该文从基因学角度探讨了*ABCA1*对人滋养细胞功能的影响。在人滋养细胞中调控*ABCA1*的表达, 通过表达谱芯片检测*ABCA1*表达改变后滋养细胞中基因及相关信号通路的改变, Western blot及qRT-PCR验证芯片结果。结果显示, 上调*ABCA1*表达时, 有197个基因表达升高, 有190个基因表达下降; 而下调*ABCA1*表达时, 有335个基因表达升高, 459个基因表达下降。GO和KEGG分析表明, *ABCA1*表达上升或下降后可导致滋养细胞内多个信号通路发生改变。qRT-PCR及Western blot检测发现, 与阴性对照组相比, *ABCA1*上调后细胞中S1PR1的mRNA及蛋白水平明显升高, 而CCL8、CXCL10与CXCL11的mRNA及蛋白水平明显降低; 下调*ABCA1*的表达后, 细胞中S1PR1的mRNA及蛋白水平明显降低, 而CCL8、CXCL10与CXCL11的mRNA及蛋白水平明显升高, 与芯片的结果完全一致。这些结果表明, *ABCA1*可通过调控多个信号通路而影响滋养细胞功能。

**关键词** *ABCA1*; 滋养细胞; 基因表达谱; 信号通路

## Effect of *ABCA1* on Gene Expression Profiles of Trophoblast Cells

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**Abstract** This article explored the effect of *ABCA1* on human trophoblast function from the genetic perspective. The expression of *ABCA1* in human trophoblast cells was regulated, and the changes of genes and related signaling pathways in trophoblast cells were detected by expression microarray, and the results were verified by Western blot and qRT-PCR. The results showed that when *ABCA1* expression was up-regulated, 197 genes were up-regulated and 190 genes were down-regulated. When *ABCA1* expression was down-regulated, 335 genes were elevated in expression and 459 genes were down-regulated. GO and KEGG analysis indicate that increased or decreased *ABCA1* expression leads to changes in multiple signaling pathways in trophoblasts. Compared with the negative control group, the mRNA and protein levels of S1PR1 in the cells up-regulated by *ABCA1* were significantly increased, while the mRNA and protein levels of CCL8, CXCL10 and CXCL11 were significantly decreased. The expression of *ABCA1* was down-regulated after qRT-PCR and Western blot assay. The mRNA and protein levels of S1PR1 in the cells were significantly decreased, while the mRNA and protein levels of CCL8, CXCL10 and CXCL11 were significantly increased, which was consistent with the results of the chip. These results indicate that *ABCA1* can affect trophoblast function by regulating multiple signaling pathways.

**Keywords** *ABCA1*; trophoblast cells; gene expression profile; signal pathways

收稿日期: 2018-11-20 接受日期: 2019-01-21

国家自然科学基金(批准号: 81471476)资助的课题

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Received: November 20, 2018 Accepted: January 21, 2019

This work was supported by the National Natural Science Foundation of China (Grant No.81471476)

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网络出版时间: 2019-05-10 16:42:19 URL: <http://kns.cnki.net/kcms/detail/31.2035.Q.20190510.1642.028.html>

Luciani等<sup>[1]</sup>在1999年首次克隆出ATP结合盒转运蛋白A1(ATP-binding cassette transport protein A1, *ABCA1*)基因,发现该基因是体内脂代谢的限速基因,主要负责将细胞内胆固醇转运至细胞外。近年来研究发现,*ABCA1*与某些妊娠期疾病的发生密切相关,可能在疾病的发生、发展过程中起到重要的作用<sup>[2]</sup>。滋养细胞作为构成胎盘的主要细胞,在维持正常的妊娠进展及各种妊娠期疾病的发病过程中发挥着重要的作用<sup>[3]</sup>。研究报道,*ABCA1*可调控滋养细胞胆固醇的转运功能,当*ABCA1*表达升高时,滋养细胞胆固醇转运量明显增强;而当*ABCA1*表达降低时,滋养细胞胆固醇转运量明显降低<sup>[4]</sup>。然而,目前关于*ABCA1*对滋养细胞中相关信号通路的影响并不明确,本文利用表达谱基因芯片技术,从基因学角度研究*ABCA1*对人滋养细胞中信号通路的影响,以探讨*ABCA1*对滋养细胞功能影响的可能机制。

## 1 材料与方法

### 1.1 材料

人滋养细胞系(HTR8-S/Vneo)由中国科学院动物所王红梅课题组提供。胎牛血清、DMEM高糖培养基购自美国Gibco公司。Trizol试剂、总RNA提取试剂盒、Fast Quant RT Kit(With gDNase)、Super-real荧光定量预混试剂盒(SYBR Green)购自美国Invitrogen公司。qRT-PCR引物由北京梓熙生物股份有限公司合成。DMEM培养基、胎牛血清、RNAiMAX转染试剂、siRNA以及Mock-siRNA、LXR $\alpha/\beta$ 泛激动剂T0901317、青霉素-链霉素双抗溶液、无菌PBS液、鼠抗人*ABCA1*单克隆抗体、兔抗人CCL8多克隆抗体、兔抗人CXCL10多克隆抗体、兔抗人CXCL11多克隆抗体、兔抗人S1PR1多克隆抗体、鼠抗人 $\beta$ -actin单克隆抗体均购自美国Sigma公司。辣根过氧化物酶(HRP)标记羊抗兔IgG抗体、辣根过氧化物酶(HRP)标记羊抗鼠IgG抗体购自北京中杉金桥生物技术有限公司。其他Western blot电泳和转膜及封闭试剂购于北京索莱宝科技有限公司。

### 1.2 方法

1.2.1 细胞培养 HTR8细胞用含10%胎牛血清的DMEM培养基培养,置于37℃、含5% CO<sub>2</sub>的培养箱中培养,细胞密度为90%左右时可进行传代,培养24~48 h传代1次。取对数生长期细胞,胰酶消化

成单细胞悬液,调整至合适的细胞密度,接种至6孔板,实验分为4组:上调*ABCA1*组(加入肝LXR $\alpha/\beta$ 泛激动剂T0901317)与对照组(加入DMSO);下调*ABCA1*组(转染siRNA)与对照组(转染Mock-siRNA)。细胞接种24 h后实验组分别加入用DMSO溶解的T0901317(使细胞培养基中的终浓度为100 nmol/L),对照组中加入相同体积的DMSO溶液,培养48 h后提取RNA;同时将配置好的RNAiMAX转染试剂以及siRNA(实验组)或Mock-siRNA(对照组)混合溶液缓慢分别加入细胞中,培养72 h后提取RNA。

1.2.2 表达谱基因芯片分析 由上海欧意生物科技有限公司经RNA提取、质量鉴定合格后,与Agilent SurePrint G3 Human Gene Expression v3(芯片容量: 8×60 000,版本号: 072363)版本的芯片进行杂交,Cy5标记aRNA,杂交程序为Agilent Scanner G2505C(Agilent Technologies)程序扫描,用Feature Extraction软件(version10.7.1.1, Agilent Technologies)处理原始图像提取原始数据。接着利用Genespring软件(version13.1, Agilent Technologies)进行quantile标准化和后续处理。

1.2.3 qRT-PCR验证芯片结果 用Trizol提取细胞总RNA,用紫外分光光度法检测总RNA纯度和浓度,取2  $\mu$ g的RNA按试剂盒说明进行反转录,取合成的cDNA进行实时荧光定量PCR,引物由Primer Premier 5.0软件设计。反应体系按试剂盒说明,反应条件为:95℃预变性15 min;95℃变性10 s,60℃退火/延伸20 s,共40个循环(使用7500-PCR Real-time PCR system荧光定量PCR仪扩增),结果分析采用2<sup>- $\Delta\Delta C_t$</sup> 法分析。

1.2.4 Western blot验证芯片结果 用SDS Lysis Buffer提取细胞总蛋白,用BCA法检测总蛋白浓度,取20  $\mu$ g蛋白样品与6×十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE)上样缓冲液按5:1比例混匀煮沸变性10 min,SDS-PAGE电泳,湿法转膜,5%牛奶封闭2 h,一抗在4℃冰箱中摇晃孵育过夜后用PBS洗膜,二抗室温孵育2 h后用PBS洗膜,化学发光法显色,胶片曝光适当时间,经显影、定影后扫描,然后用Image J软件读取各组条带灰度值进行统计分析。

1.2.5 统计分析 所有的实验数据采用SPSS 15.0软件进行统计分析。计量资料以 $\bar{x}\pm s$ 表示,组间比较采用*t*检验,以*P*<0.05认为差异有统计学意义。

## 2 结果

### 2.1 滋养细胞中*ABCA1*调控效果验证

应用qRT-PCR及Western blot验证滋养细胞中*ABCA1*表达量的调控效果,加入肝LXR $\alpha/\beta$ 泛激动剂T0901317后滋养细胞中*ABCA1*表达量较对照组明显升高( $P<0.05$ );转染siRNA滋养细胞*ABCA1*的表达量明显较对照组明显降低( $P<0.05$ ),结果如图1所示,达到我们做基因芯片的要求。

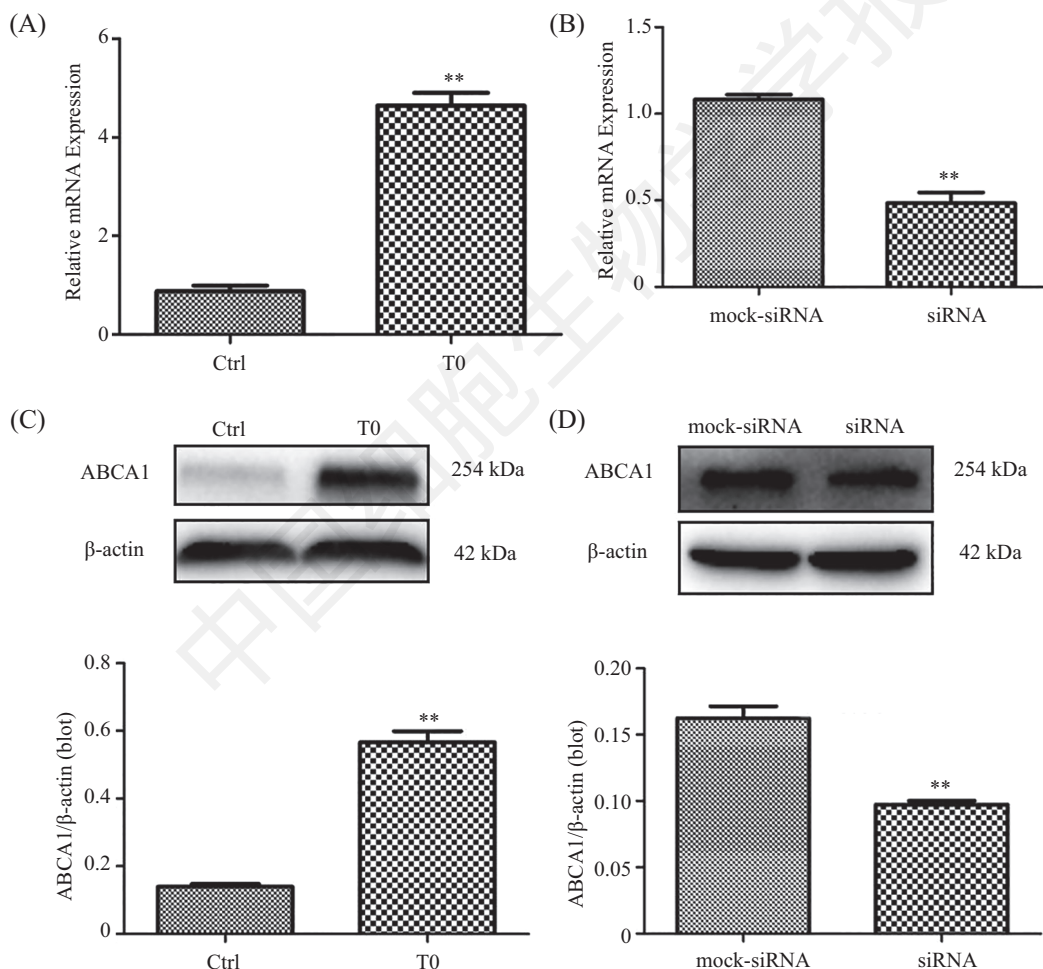
### 2.2 *ABCA1*表达改变后滋养细胞中表达发生改变的基因

当*ABCA1*表达改变后滋养细胞中多个基因表达发生明显改变,我们以表达改变量 $>2$ 倍且 $P<0.05$

认为改变有统计学意义为标准进行筛选<sup>[5]</sup>。当*ABCA1*表达升高时,下游有197个基因表达升高,另外有190个基因表达下降。而当*ABCA1*表达量下降时,下游有335个基因表达升高,另外有459个基因表达下降,结果如图2所示。

### 2.3 *ABCA1*表达改变导致下游发生改变的基因的表达情况

当*ABCA1*表达改变后滋养细胞中多个基因表达发生明显改变,我们以表达改变量 $>2$ 倍且 $P<0.05$ 认为改变有统计学意义为标准进行筛选<sup>[5]</sup>。当*ABCA1*表达升高时,下游有197个基因表达升高,另外有190个基因表达下降。而当*ABCA1*表达量下降



A: qRT-PCR验证上调滋养细胞*ABCA1*表达量的结果; B: qRT-PCR验证下调滋养细胞*ABCA1*表达量的结果; C: Western blot验证上调滋养细胞*ABCA1*表达量的结果; D: Western blot验证下调滋养细胞*ABCA1*表达量的结果。Ctrl: 对照组; T0: LXR $\alpha/\beta$ 泛激动剂T0901317组; mock-siRNA: 转染空载体组(对照组); siRNA: 转染siRNA组; \*\* $P<0.01$ , 与对应的对照组比较,  $n=5$ 。

A: qRT-PCR confirmed the up-regulation of trophoblastic *ABCA1* expression; B: qRT-PCR confirmed down-regulation of trophoblastic *ABCA1* expression; C: Western blot confirmed up-regulation of trophoblastic *ABCA1* expression; D: Western blot verified the results of down-regulating the expression of *ABCA1* in trophoblast cells. Ctrl: control group; T0: LXR $\alpha/\beta$  panagonist T0901317 group; mock-siRNA: transfected empty plasmid group (control group); siRNA: transfected siRNA group; \*\* $P<0.01$  vs corresponding control group,  $n=5$ 。

图1 qRT-PCR及Western blot验证滋养细胞中*ABCA1*的调控效果

Fig.1 qRT-PCR and Western blot were used to verify the regulatory effect of *ABCA1* in trophoblasts

时,下游有335个基因表达升高,另外有459个基因表达下降。我们用热图软件Mev,采用的聚类方法为分层聚类距离评估方式为pearson相关系数对这些表达改变的基因进行关联分析。图3为*ABCA1*表达改变导致下游发生改变的基因的表达热图,蓝色代表低表达黄色代表高表达。热图使用软件为Mev,聚类方法为分层聚类距离评估方式为pearson相关系数。

#### 2.4 滋养细胞中表达发生改变的基因的功能富集分析

用DAVID软件分析*ABCA1*表达改变后导致滋养细胞中表达发生改变的基因功能,统计方法为卡方检验。生物过程分析表明,差异表达基因参与了众多信号通路的表达与调控等,其中主要参与了趋化因子的应答过程;而对于细胞组分分析表明,差异表达基因主要参与细胞外空间成分的表达;分子功能富集分析发现,差异表达基因主要富集于肝素结合和趋化因子活性。结果如图4所示。

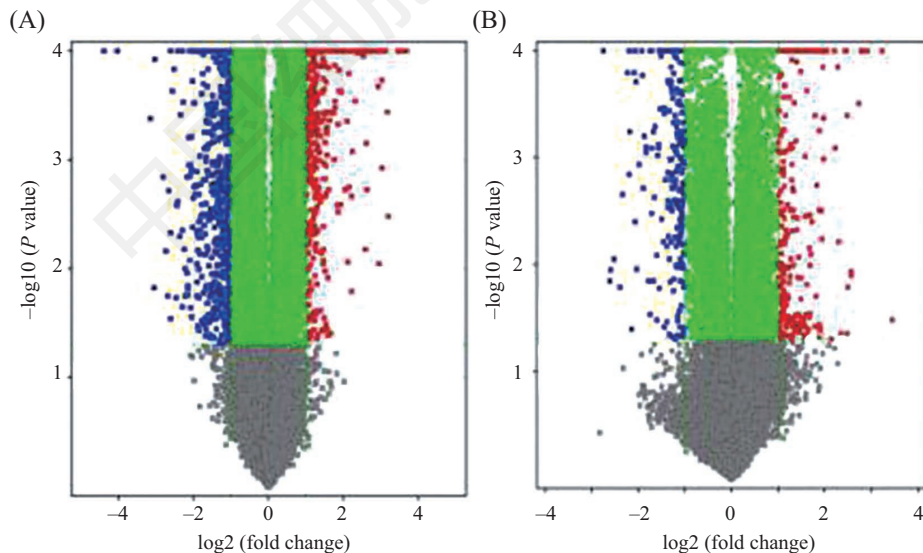
#### 2.5 qRT-PCR验证芯片结果

在所有表达发生改变的基因中,我们从中挑选出*CCL8*、*CXCL10*、*CXCL11*以及*SIP1R1*等四个基因作为代表基因,用qRT-PCR验证芯片结果的可靠性。

用Trizol将经过LXR $\alpha/\beta$ 泛激动剂T0901314处理过的细胞及转染过siRNA的细胞提取总RNA,应用qRT-PCR检测*ABCA1*、*CCL8*、*CXCL10*、*CXCL11*以及*SIP1R1*的表达情况发现,与对照组相比,经LXR $\alpha/\beta$ 泛激动剂T0901314处理后的细胞中*ABCA1*及*SIP1R1*的mRNA水平明显升高,而*CCL8*、*CXCL10*与*CXCL11*的mRNA水平明显降低( $P<0.01$ ,图5A);而转染过siRNA后的细胞中*ABCA1*及*SIP1R1*的mRNA水平明显降低,而*CCL8*、*CXCL10*与*CXCL11*的mRNA水平明显升高( $P<0.01$ ,图5B),结果与芯片的结果完全一致。

#### 2.6 Western blot验证芯片结果

用SDS裂解液提取用LXR $\alpha/\beta$ 泛激动剂T0901314处理过的细胞及转染过siRNA的细胞的总蛋白,应用Western blot检测所挑选出的*ABCA1*、*CCL8*、*CXCL10*、*CXCL11*以及*SIP1R1*的蛋白表达情况发现,与对照组相比,经LXR $\alpha/\beta$ 泛激动剂T0901314处理后的细胞中*ABCA1*及*SIP1R1*的蛋白水平明显升高,而*CCL8*、*CXCL10*与*CXCL11*的蛋白水平明显降低( $P<0.01$ ,图6A和图6B);转染过siRNA后的细胞中,*ABCA1*及*SIP1R1*的蛋白水平明显降低,而*CCL8*、*CXCL10*与*CXCL11*的蛋白水平明显升高( $P<0.01$ ,图6C和图6D),结果与芯片的结果完全一致。

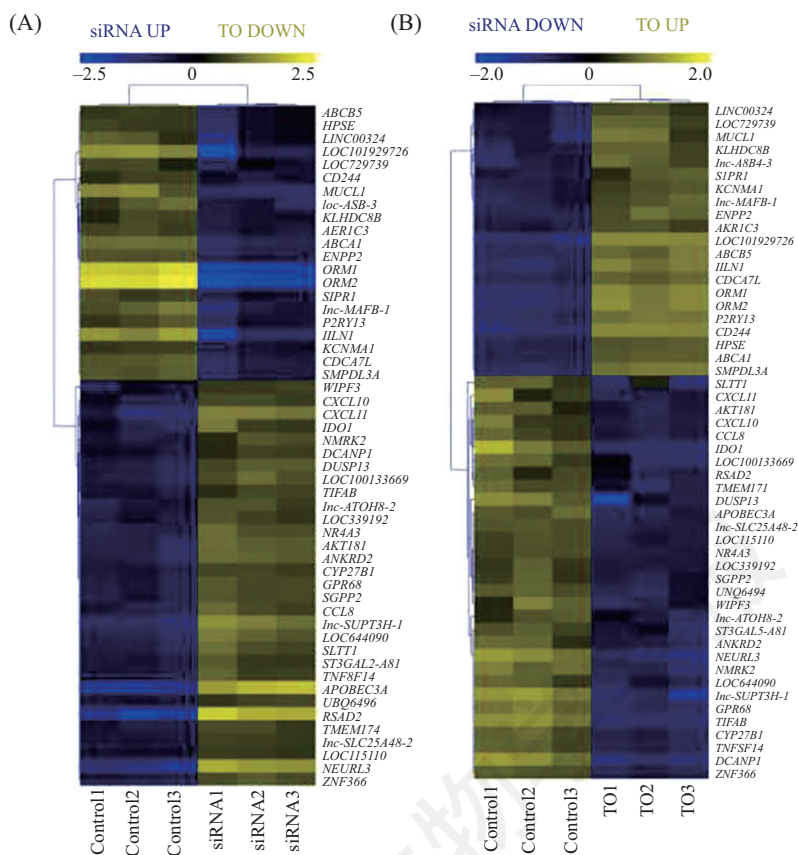


A: *ABCA1*表达升高后下游基因的改变数目的火山图; B: *ABCA1*表达下降后下游基因的改变数目的火山图。蓝色代表表达降低超过2倍且 $P<0.05$ 的基因,红色代表表达升高超过2倍且 $P<0.05$ 的基因,绿色代表表达改变小于2倍且 $P<0.05$ 的基因,灰色代表表达改变小于2倍的基因。

A: volcano map of the number of altered genes in downstream genes after *ABCA1* expression been elevated; B: volcano map of the number of altered genes in downstream genes after *ABCA1* expression been decreased. Blue represents the genes with a decrease in expression more than 2 fold and  $P<0.05$ , red represents the genes with an expression increase of more than 2 fold and  $P<0.05$ , green represents the genes with a change of expression less than 2 fold and  $P<0.05$ , and gray represents genes with expression changes less than twice.

图2 *ABCA1*表达发生改变后滋养细胞中基因表达发生改变数目的火山图

Fig.2 Volcano map showing the number of changes in gene expression in trophoblast cells after *ABCA1* expression changes



蓝色代表低表达,黄色代表高表达。热图使用软件为Mev,聚类方法为分层聚类距离评估方式为pearson相关系数。

Blue represents low expression and yellow represents high expression. The software is Mev, and the clustering method is pearson correlation coefficient.

图3 *ABCA1*表达改变导致下游发生改变的基因的表达热图

Fig.3 The expression thermogram of genes downstream from *ABCA1* expression changes

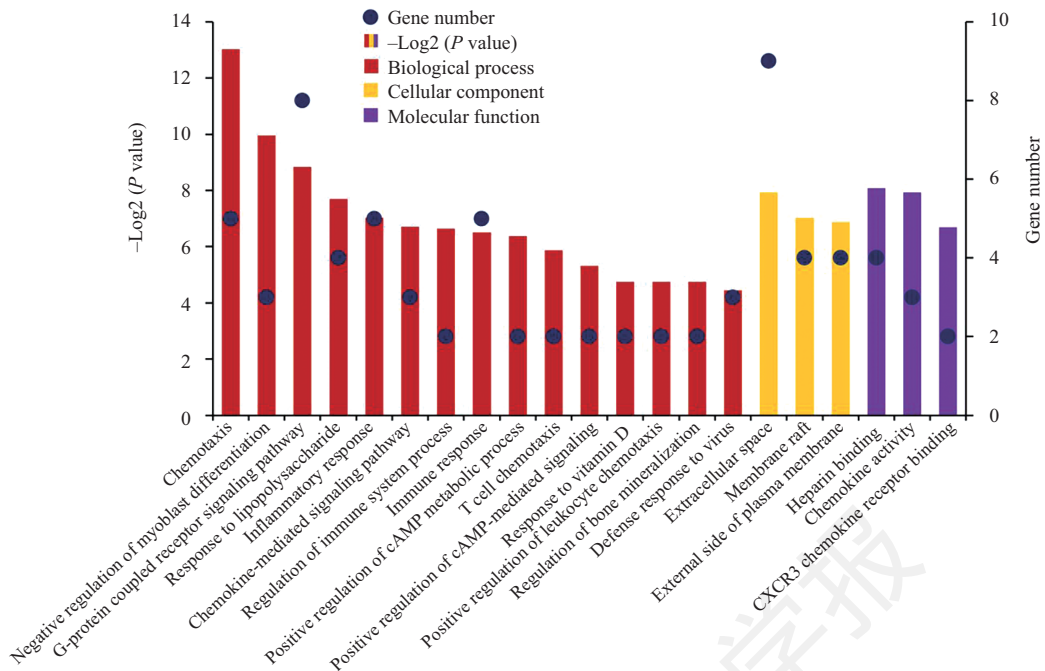
### 3 讨论

胎盘滋养细胞约在受精后的第4天左右出现,到孕中期时分化成两种类型:细胞滋养细胞以及合体滋养细胞。合体滋养细胞直接与母血接触,而细胞滋养细胞可进一步分为绒毛外滋养细胞、绒毛内滋养细胞以及血管内滋养细胞<sup>[6-7]</sup>。滋养细胞作为构成胎盘的主要成分,其功能的改变可使得胎盘功能发生显著改变<sup>[4]</sup>。胎盘滋养细胞在妊娠期是胎盘中最活跃的细胞,滋养细胞层内部复杂的结构及功能使得胎盘具有母胎界面屏障作用、内分泌、物质交换以及免疫耐受等功能,当滋养细胞功能失调后可导致一系列妊娠期疾病的发生。滋养细胞在接受内部或外界的各种信号刺激后,经过各种激酶等相关信号通路转导,最后导致滋养细胞功能及活性等发生相应改变。

我们的研究发现,当滋养细胞中*ABCA1*表达改变后,滋养细胞基因表达谱发生明显变化,众多基因的表达水平发生改变。进一步行富集分析发

现,滋养细胞中表达发生改变的基因参与了众多的细胞生物学过程、分子构成以及细胞组分等;同时发现滋养细胞中CCL8、CXCL10、CXCL11以及S1PR1的表达量随之发生改变。当*ABCA1*表达升高时,S1PR1表达量随之升高,而CCL8、CXCL10以及CXCL11表达量明显降低。当*ABCA1*表达降低时,S1PR1表达量随之降低,而CCL8、CXCL10以及CXCL11表达量明显升高,并且qRT-PCR与Western blot结果验证了表达谱芯片结果的可靠性。结合既往文献的报道<sup>[8]</sup>,我们认为在滋养细胞中,*ABCA1*可与S1PR1相互结合,使得S1PR1受体被激活,特异性的与G蛋白偶联受体结合,导致下游一系列信号通路的激活,使细胞分泌的趋化因子CCL8、CXCL10以及CXCL11水平发生改变,参与免疫应答的调控。当滋养细胞免疫功能发生改变时,可导致胎盘功能随之发生改变,进而影响妊娠过程的进行。

趋化因子超家族是一类在机体处于炎症或病理状态时引导粒细胞定向转运的可溶性小蛋白分

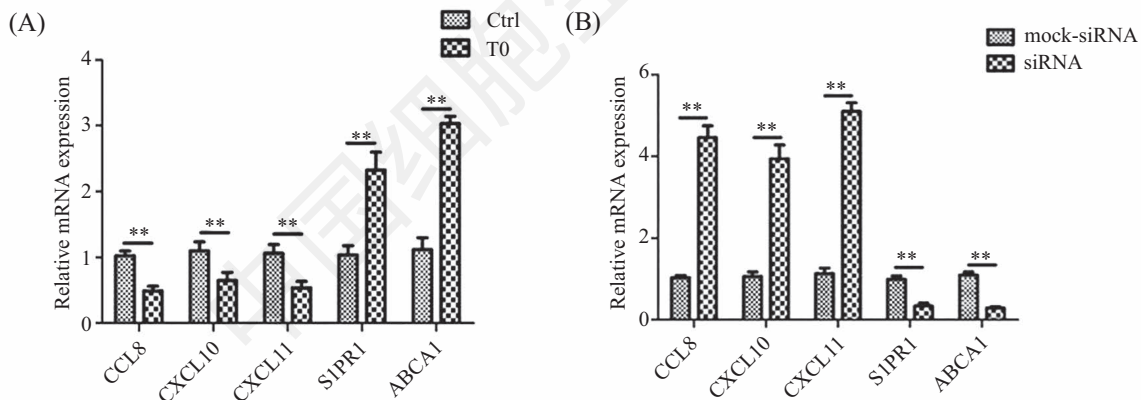


*ABCA1*表达改变后导致滋养细胞中表达发生改变的基因功能的结果,所用软件为DAVID软件,统计方法为卡方检验。

The result of changes in the expression of *ABCA1* leading to altered gene expression in trophoblast. The software was DAVID, and the statistical method was chi-square test.

图4 滋养细胞中表达发生改变的基因的功能富集分析

Fig.4 Functional enrichment analysis of genes with altered expression in trophoblast



A: 经LXR $\alpha/\beta$ 激动剂T0901314处理后的细胞中*ABCA1*、*CCL8*、*CXCL10*、*CXCL11*及*SIP1R1*的mRNA表达情况; B: 转染过siRNA后的细胞中*ABCA1*、*CCL8*、*CXCL10*、*CXCL11*及*SIP1R1*的mRNA。 \*\* $P < 0.01$ ,  $n = 5$ 。

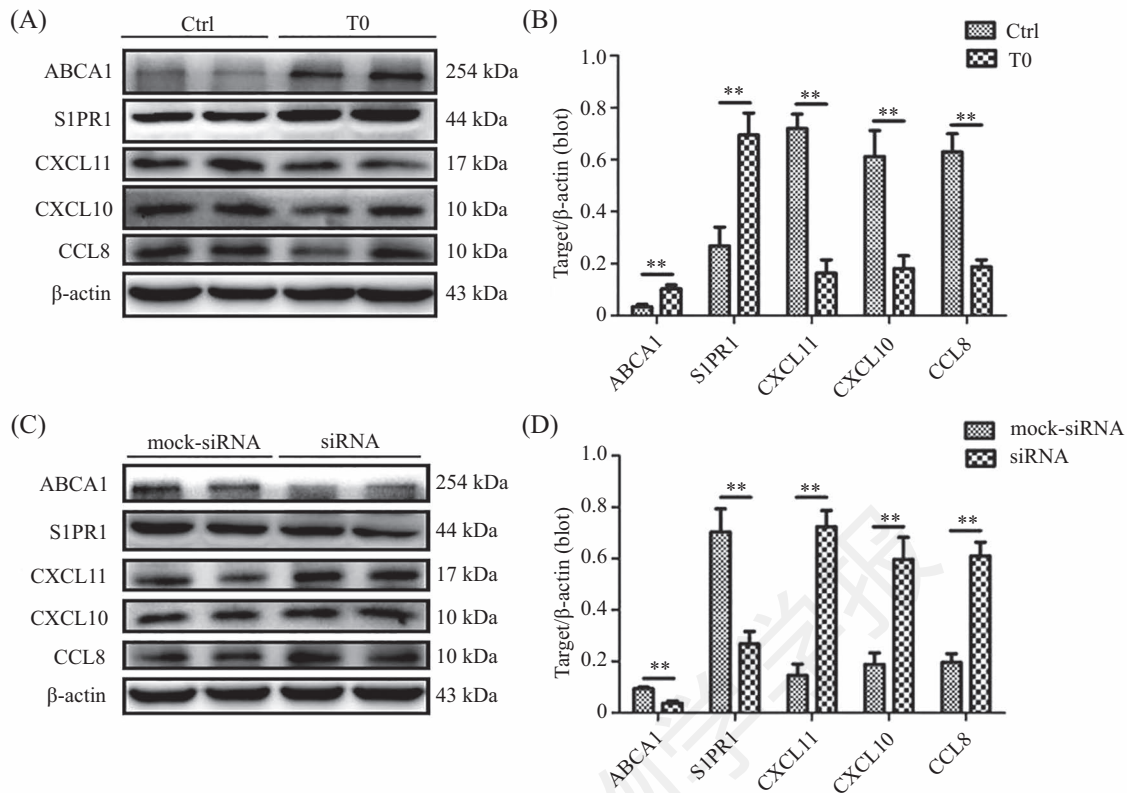
A: the expression of *ABCA1*, *CCL8*, *CXCL10*, *CXCL11* and *SIP1R1* in cells treated with T0901314; B: the expression of *ABCA1*, *CCL8*, *CXCL10*, *CXCL11* and *SIP1R1* in cells transfected with siRNA. \*\* $P < 0.01$ ,  $n = 5$ 。

图5 qRT-PCR验证基因芯片结果

Fig.5 qRT-PCR validates the results of gene chip

子,在免疫应答以及胚胎发育过程中起到重要的作用<sup>[7]</sup>。根据趋化因子分子结构中的半胱氨酸残基的数量以及其在空间位置上的排列顺序差异,趋化因子可分为4大类: CXC( $\alpha$ )、CC( $\beta$ )、XC( $\gamma$ )和CX3C( $\delta$ )趋化因子。大部分趋化因子属于G蛋白偶联受体,例如CCL8、CXCL10、CXCL11等都为典型受体;

有少部分属于非G蛋白偶联受体,为非典型受体,包括D6、DARC、CCRL1和CXCR7<sup>[9]</sup>。G蛋白偶联受体被激活后,可导致下游一系列信号通路的激活,包括MAPK、RAS、Rho GTPases和PI3K等,这些通路被激活后可直接影响中性粒细胞的迁移和增殖;同时,通过激活蛋白激酶B(PKB/AKT)通路和上调抗调



A, B: 经LXR $\alpha$ / $\beta$ 泛激动剂T0901314处理后的细胞中ABCA1、CCL、CXCL10及S1PR1的表达情况; C、D: 转染siRNA后的细胞中ABCA1、CCL8、CXCL10、CXCL11及S1PR1的表达情况。 \*\* $P < 0.01$ ,  $n = 5$ 。  
A,B: the expression of ABCA1, CCL8, CXCL10, CXCL11 and S1PR1 in cells treated with T0901314; C,D: the expression of ABCA1, CCL8, CXCL10, CXCL11 and S1PR1 in cells transfected with siRNA. \*\* $P < 0.01$ ,  $n = 5$ .

图6 Western blot验证基因芯片结果

Fig.6 Western blot validates microarray results

亡基因的表达可以促进细胞的存活;此外,这些受体还可以反向激活其他信号通路(如Jak-STATs)及受体(如酪氨酸激酶受体),从而发挥特定的免疫调节作用<sup>[10]</sup>。一种趋化因子可以结合多个受体,同时一个受体也可以被多种趋化因子所激活,这种配体与受体结合的复杂性决定了趋化因子生物学功能的多样性<sup>[11]</sup>。当前的研究认为,趋化因子及其受体不仅参与炎症时粒细胞的迁移,而且与免疫细胞成熟与运输以及淋巴组织的形成密切相关。另外,趋化因子及其受体表达异常在机体多种疾病的发生、发展过程中起到了重要的作用,比如自身免疫性疾病、炎症性疾病以及肿瘤等<sup>[12-14]</sup>。

综上所述, *ABCA1*表达改变后可诱导滋养细胞中众多基因表达发生明显变化,进而使得这些基因所具备的功能、对应的信号通路及分子机制等发生改变,导致滋养细胞的生物学功能出现异常,从而导致各种滋养细胞相关疾病的发生。

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