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腰椎间盘脱出症非手术治疗前后外周血基因表达变化特征及意义*

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【摘要】 目的 研究腰椎间盘脱出患者外周血基因表达特征及非手术治疗对其表达的影响。**方法** 采用基因芯片半定量测定初步筛选腰椎间盘脱出患者和健康对照者外周血中的差异表达基因，以及患者在非手术治疗后这些差异表达基因的变化趋势，通过富集分析研究差异表达基因的功能特征，通过网络分析找出基因异常表达的关键基因，采用qRT-PCR定量检测验证这些基因在患者和健康对照样本中的表达情况以及非手术治疗对这些差异表达基因的影响。**结果** 在腰椎间盘脱出患者和健康对照组的外周血中发现153个差异表达基因，其中131个基因表达上调，22个基因表达下调；富集分析显示大部分差异表达基因与免疫以及炎症反应相关；网络分析显示Toll样受体4（toll-like receptor 4, *TLR*4）、基质金属肽酶9（matrix metallopeptidase 9, *MMP*9）、髓过氧化物酶（myeloperoxidase, *MPO*）、抗菌肽（cathelicidin antimicrobial peptide, *CAMP*）、resistin基因（*RETN*）和Toll样受体5（toll-like receptor 5, *TLR*5）是蛋白互作网络中的关键基因，这些关键基因均被富集到了免疫、炎症反应相关的条目。非手术治疗后，患者疼痛减轻，在这153个差异表达基因中，*TLR*5、白介素1受体拮抗剂（interleukin 1 receptor antagonist, *IL*1*RN*）和溶质载体家族8成员A1（solute carrier family 8 member A1, *SLC*8*A*1）在治疗后表达下调。qRT-PCR结果显示：患者外周血中*TLR*4、*MMP*9、*MPO*、*CAMP*、*RETN*、*TLR*5、*IL*1*RN*和*SLC*8*A*1表达水平高于健康对照组（$P<0.05$）；治疗后与治疗前比较，*TLR*5、*IL*1*RN*和*SLC*8*A*1表达水平降低（$P<0.05$）。**结论** 腰椎间盘脱出患者外周血基因表达特征主要是免疫和炎症反应相关基因表达失调，其中*TLR*4、*MMP*9、*MPO*、*CAMP*、*RETN*和*TLR*5这些与免疫和炎症反应相关的基因在腰椎间盘脱出患者外周血基因表达失调中起关键作用，非手术治疗疗效的获得可能与患者外周血中过度表达的*TLR*5、*IL*1*RN*和*SLC*8*A*1下调相关。

【关键词】 腰椎间盘脱出 外周血 基因差异表达 非手术治疗

Characteristics and Significance of Gene Expression Changes in Peripheral Blood of Lumbar Disc Extrusion Patients before and after Nonoperative Treatment

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【Abstract】 Objective To define the gene expression characteristics in the peripheral blood of patients with lumbar disc extrusion (LDE) and the effect of nonoperative treatment on the gene expression. **Methods** DNA microarray was used to identify semi-quantitatively the differentially expressed genes (DEGs) in the peripheral blood of patients with LDE and that of the healthy controls and the variation trend of these DEGs after nonoperative treatment. Enrichment analysis was done to reveal the functional characteristics of these DEGs, and network analysis was done to identify key genes that contribute to gene dysregulation. The levels of these key genes were measured by qRT-PCR to examine their expression in LDE patients and the controls, and the effect of nonoperative treatment on the expression level. **Results** We identified 153 DEGs in the peripheral blood of LDE patients and healthy controls, including 131 upregulated genes and 22 downregulated genes. Enrichment analysis revealed that most of the DEGs were related to immunity and the inflammatory response. Network analysis revealed that toll-like receptor 4 (*TLR*4), matrix metallopeptidase 9 (*MMP*9) and myeloperoxidase (*MPO*), cathelicidin antimicrobial peptide (*CAMP*), resistin (*RETN*), toll-like receptor 5 (*TLR*5) were the key genes in the protein-protein interaction network. These key genes were all enriched into the terms releated to immunity and the inflammatory response. The patients experienced pain relief after nonoperative treatment. Among the 153 DEGs, *TLR*5 , interleukin 1 receptor antagonist (*IL*1*RN*) and solute carrier family 8 member A1 (*SLC*8*A*1) were downregulated after nonoperative treatment. qRT-PCR revealed that the levels of *TLR*4, *MMP*9, *MPO*, *CAMP*, *RETN*, *TLR*5, *IL*1*RN* and *SLC*8*A*1 in the peripheral blood of the LDE patients were higher than those of the healthy control group ($P<0.05$). In addition, *TLR*5, *IL*1*RN* and *SLC*8*A*1 expression levels decreased after treatmentin comparison with the levels before treatment ($P<0.05$). **Conclusion** Gene expression in the peripheral blood of LDE patients was characterized by the dysregulation of immune and inflammatory response-related genes,

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among which, *TLR*4, *MMP*9, *MPO*, *CAMP*, *RETN* and *TLR*5, the genes relevant to immune and inflammatory response, played a key role in the dysregulation of gene expression in the peripheral blood of LDE patients. The outcome of non-operative treatment may be related to the downregulation of the overexpressed *TLR*5, *IL*1*RN* and *SLC*8*A*1 in the peripheral blood of patients.

【Key words】 Lumbar disc extrusion　Peripheral blood　Differential gene expression　Nonoperative treatment

腰椎间盘突出症引起下肢神经痛被认为是机械压迫、免疫反应和炎症反应共同作用的结果,手术治疗可缓解神经根的机械压迫,但在术后仍有不少患者存在不同程度的疼痛和麻木[1],同时临床上也存在大量椎间盘突出而无症状人群,在非手术治疗后突出物依然存在而症状缓解,因此机械因素只是导致症状的条件之一。随着分子生物学的进展,髓核的化学刺激和免疫反应在腰腿痛的作用受到越来越多的重视[2-3]。

目前对腰椎间盘突出症的分型方法较多[3],椎间盘脱出是指纤维环全层破裂,髓核从纤维环裂口脱出,本研究所指腰椎间盘脱出包括美国密歇根州立大学(Michigan State University, MSU)分型[4]的2级和3级。临床发现脱出型腰椎间盘突出症(lumbar disc extrusion, LDE)患者的症状通常比包容型的症状更严重、更持久,在局麻下行此类腰椎间盘突出手术时发现神经根明显充血水肿,用神经剥离子轻轻触碰神经根能引起患者剧烈的下肢痛,若症状反复发作,神经根周围粘连就更加严重[5-6]。

腰椎间盘突出是一种局灶性病变,目前的研究多集中在突出椎间盘局部。既往的研究发现肿瘤坏死因子α(tumor necrosis factor α, TNF-α)、白细胞介素(interleukin, IL)-4、IL -6、IL -8、IL -10、IL -17、IL -21、T辅助细胞(Th)17、磷脂酶A2、C反应蛋白C-X3-C基序配体1、C-C基序配体2和肥大细胞蛋白酶1等免疫和炎症反应相关因子在腰椎间盘突出症患者外周血中表达异常[7-14]。这些研究以外周血中单个或多个因子为研究对象,缺乏在整体上对外周血基因表达的观测。另一方面,不同类型椎间盘突出引起的炎症和免疫反应程度不同,椎间盘脱出比包容型引起的免疫和炎症反应程度通常更重,既往的研究大多没有区分椎间盘突出的类型。因此本研究着眼于LDE患者,从整个基因表达谱层面探索LDE患者外周血基因表达特征,并初步研究非手术治疗潜在的作用机制。

1　对象与方法

1.1　研究对象

2018年4月–2019年12月,我们按研究计划连续选取25例住院接受我科规范化非手术治疗的LDE患者为研究对象,并招募25例健康志愿者作为对照组。病例纳入标准:①年龄:18 ~ 60岁;②按北美脊柱协会(North American Spine Society, NASS)推荐诊疗指南[15],满足LDE的影像学描述,并有与之相吻合的临床征象;③非手术治疗有效,包括适当的卧床休息、物理治疗、必要的止痛药、推拿、针灸、生活方式的修正及适宜的身体训练等,所有治疗均按我院标准操作规范进行。排除标准:①肿瘤、骨质疏松、创伤、各种滑脱、狭窄、感染、侧凸等畸形;②神经肌肉性、代谢性、风湿免疫性疾病;③循环系统、内分泌系统疾病;④放、化疗史,输血史,器官移植史;⑤1年内手术史;⑥既往脊柱手术史;⑦妊娠或哺乳期妇女;⑧超过一个节段的突出。健康志愿者纳入标准:①年龄:18 ~ 60岁;②无椎间盘突出、肿瘤、骨质疏松、创伤、各种滑脱、狭窄、感染、侧凸等畸形;③无神经肌肉性、代谢性、风湿免疫性疾病;④无循环系统、内分泌系统疾病;⑤无放、化疗史,无输血史,无器官移植史;⑥无原因不明的疼痛;⑦1年内未接受任何手术;⑧1年内无妊娠、哺乳;⑨意识清醒,无精神障碍,无智力障碍,无身体残疾。本研究经四川省骨科医院伦理委员会批准,所有参与者自愿参加并签署知情同意文件。

1.2　血液样本采集和保存

所有研究对象于早上7:00–7:30间从肘部静脉采集空腹血液10 mL,患者血液采集时间为治疗前和治疗第15天,对照组血液与患者治疗前同期采集。血液标本保存在PAX全血RNA管(BD, Biosciences)内,–20 °C冷冻保存。

1.3　主要试剂

芯片试验试剂:Agilent低输入快速扩增标记试剂盒(One-Color Low Input Quick Amp Labeling Kit),Agilent表达谱芯片配套试剂盒(Gene Expression Hybridization Kit),Agilent 配套洗片试剂盒(Gene Expression Wash Buffer Kit)。实时荧光定量PCR(qRT-PCR)试剂:TOYOBO逆转录试剂盒(ReverTra Ace qPCR Kit),ABI荧光定量PCR预混液(Power SYBR Green),Ambion非DEPC处理无核酸水。

1.4　RNA的抽提与纯化

采用Qiagen PAXgene™ Blood RNA Kit进行样品的总

RNA抽提后使用Agilent Bioanalyzer 2100检测样品总RNA质量,合格后通过Agilent NanoDrop ND-2000分光光度计进行核酸浓度定量,然后采用Agilent低输入快速扩增标记试剂盒对样品总RNA进行放大和标记,并用Qiagen总RNA提取试剂盒(RNeasy mini kit)纯化标记后的cRNA,再用Agilent表达谱芯片配套试剂盒在滚动杂交炉中以65 ℃、10 r/min滚动杂交17 h,杂交cRNA上样量1.65 μg,并在洗缸中用Agilent 配套洗片试剂盒试剂洗片,最后用Agilent Microarray Scanner扫描完成杂交的芯片,软件设置Dye channel: Green, Scan resolution= 3 μm, PMT 100%, 20 bit。用Feature Extraction software 10.7读取数据。

1.5 芯片实验及数据预处理

芯片实验使用安捷伦SurePrint G3 (8x60K)人类基因表达微阵列,并遵照安捷伦公司的标准操作手册执行。芯片扫描得到的原始数据由R软件中limma包进行归一化处理,所用的算法为Quantile,归一化信号值为经过log2计算的信号值。

1.6 筛选差异表达基因(differentially expressed genes, DEGs)

先删除不能识别的探针对应的数据,多个探针对应相同基因时则计算多个探针测的表达数据均值。用R语言从归一化后的基因表达谱数据中筛选DEGs,采用差异倍数(fold change, FC)≥1.5以及*t*检验(Student's *t*-test)*P*值< 0.05对DEGs进行筛选。

1.7 富集分析

富集分析使用Metascape(http://metascape.org)网站进行[16],包括基因本体(gene ontology, GO) 分析以及京都基因和基因组数据库(Kyoto Encyclopedia of Genes and Genomes, KEGG)通路富集分析。GO分析包括生物过程(biological processes, BP)、细胞成分(cellular components, CC)和分子功能(molecular functions, MF)。

1.8 蛋白互作(protein-protein interaction, PPI)网络

将差异基因列表上传至STRING(Search Tool for the Retrieval of Interacting Genes, https://string-db.org/)数据库,利用该数据库在线工具计算基因间的结合分,将结合分大于0.4的基因构建PPI网络,在Cytoscape(V 3.6.1)软件中将PPI网络可视化,利用CentiScaPe插件计算每个DEG的中心度数,利用MCODE插件筛选关键子网络,关键子网络筛选参数设置:“degree cutoff= 2”,“node score cutoff= 0.2”,“k-core= 2”and“max depth= 100”。

1.9 qRT-PCR

关键差异基因在所有样本中的表达量使用QuantStudio 5 Real-Time PCR 系统(ABI, USA),采用$2^{-\Delta\Delta Ct}$法用qRT-PCR定量测定,内参基因选择 β-actin。引物采用Primer Express 3.0.1软件设计,引物序列见表1。首先获取cDNA第一链,反应体系包括5×RT Buffer 4 μL, Enzyme Mix 1 μL, Primer Mix 1 μL, RNA template (0.5 μg) + H_2O 14 μL。RNA加入 0.2 mL PCR 管中配制反应溶液后将PCR 管置于 PCR 仪上,运行程序如下: 37 ℃, 15 min; 98 ℃, 5 min; 4 ºC。进行qPCR反应,反应体系包括: 2×SYBR Green PCR buffer 17.5 μL, Forward primer (10 μmmol/L) 1.75 μL, Reverse primer (10 μmmol/L) 1.75 μL, cDNA Template(5 ng)3.5 μL, H_2O 10.5 μL。将9 μL基因特异性qPCR反应液和1 μL样品加入384孔板中置于QuantStudio 5 Real-Time PCR 系统中,用QuantStudio™ Design & Analysis 软件运行程序: 50 ℃, 2 min; 95 ℃, 10 min; (95 ℃, 15 s; 60 ℃, 1 min)40个循环。最后添加熔解曲线。

表1 实时荧光定量PCR引物序列

Table 1 Sequences of primers used for quantitative real-time polymerase chain reaction (qRT-PCR)

Gene	Sequence (5′ to 3′)
TLR4	F: CCTGAGGCATTTAGGCAGCTA
	R: GATAAATCCAGCACCTGCAGTTC
MMP9	F: CACGCACGACGTCTTCCA
	R: AAGCGGTCCTGGCAGAAAT
MPO	F: CGGTACCCAGTTCAGGAAGCT
	R: CCCTCGTTCTCCCACCAAA
CAMP	F: TCAAGGATTTTTTGCGGAATCT
	R: GCCAGGGTAGGGCACACA
RETN	F: AGCCATCAATGAGAGGATCCA
	R: AGGCCAATGCTGCTTATTGC
TLR5	F: TCTGCTAGGACAACGAGGATCA
	R: CCATGAGCACCACTCCTAGGA
IL1RN	F: CAGCTGGAGGCAGTTAACATCA
	R: GAAGCGCTTGTCCTGCTTTC
SLC8A1	F: CCAGACACATTTGCCAGCAA
	R: CTATGGAGGCGTCTGCATACTG
β-actin	F: CTGGAACGGTGAAGGTGACA
	R: CGGCCACATTGTGAACTTTG

F: Forward; R: Reverse.

2 结果

2.1 患者基本资料和疼痛评分

本研究共招募了25例在我科接受非手术治疗的患

者, 所有患者均符合上述腰椎间盘突出症诊断标准, 腰椎核磁共振证实椎间盘突出分型为MSU分型2级或3级, 19～54岁(平均40岁), 男18例, 女7例, 25例健康志愿者: 19～30岁(平均23岁), 男12例, 女13例。患者视觉模拟评分(visual analogue scale, VAS)治疗前为6～8分(平均7.4分), 治疗后为0～3分(平均2.1分), 治疗前后VAS评分差异有统计学意义($P<0.05$), 即患者疼痛明显缓解, 病情好转。

2.2　基因芯片实验结果

本研究在患者和健康对照者的外周血标本中共筛选出153个DEGs(FC绝对值最大的前10个DEGs见表2)。与健康对照组比较, 患者组治疗前外周血中131个基因表达上调, 22个基因表达下调。在这153个差异基因中, Toll样受体5(Toll-like receptor 5, *TLR*5)、白介素1受体拮抗剂(interleukin 1 receptor antagonist, *IL*1*RN*)和溶质载体家族8成员A1(solute carrier family 8 member A1, *SLC*8*A*1)在非手术治疗后表达下调(表3)。

2.3　富集分析结果

GO富集分析显示治疗前和健康对照组比较上调的差异表达基因富集在115个BP条目、25个CC条目和4个MF条目(图1), 下调的差异表达基因富集在10个BP条目和1个MF条目(图2)。KEGG通路富集分析显示这些差异表达基因富集在12条KEGG通路(图3), 这些差异表达基因所显著富集的条目多数与免疫和炎症反应相关, 表明LDE患者外周血基因表达以免疫和炎症反应相关基因的异常表达为特征。

2.4　PPI网络

77个节点和255条边构成了治疗前和健康对照组比较差异表达基因的PPI网络 (图4)。由Cytoscape软件CentiScaPe插件计算出中心度数最高的15个DEGs如表4所示, 包括*TLR*4(中心度26)、*MMP*9(中心度22)、*MPO*(中心度20)、*CAMP*(中心度18)、*RETN*(中心度18)、

表 2　腰椎间盘脱出患者和健康对照者外周血样本之间表达上调和下调差异倍数最大的10个差异表达基因

Table 2　The 10 differentially expressed genes (DEGs) with the largest fold change of up-regulation and down-regulation between peripheral blood samples of patients with lumbar disc extrusion (LDE) and the control group

Gene	*P*	Fold change	Function
Up-regulated			
*PRDM*8	0.01	3.56	DNA binding, protein binding, methyltransferase activity, transferase activity, metal ion binding.
*OLFM*4	0.03	3.15	Catalytic activity, structural molecule activity, protein binding, cadherin binding.
*RPGRIP*1	0.03	3.00	Protein binding, extracellular matrix structural, constituent, protein binding, DNA binding
*COL9A*2	0.02	2.84	Extracellular matrix structural constituent, protein binding, extracellular matrix structural constituent conferring tensile strength
*BATF*2	0.02	2.81	RNA polymerase Ⅱ proximal promoter sequence-specific DNA binding, DNA-binding transcription factor activity, RNA polymerase Ⅱ-specific
*FCGR*1*A*	<0.001	2.53	Transmembrane signaling receptor activity, protein binding, IgG binding
*CEACAM*8	0.02	2.42	Protein binding, protein heterodimerization activity
LTF	<0.001	2.41	Lipopolysaccharide binding, DNA binding, serine-type endopeptidase activity, iron ion binding
*LCN*2	0.02	2.24	Protease binding, iron ion binding, protein binding, small molecule binding, identical protein binding
*DEFA*4	0.02	2.15	Protein homodimerization activity
Down-regulated			
*PLXDC*1	0.01	0.63	Protein binding
*CD*160	<0.001	0.63	Transmembrane signaling receptor activity, signaling receptor binding, protein binding, kinase binding
*KLRC*3	<0.001	0.63	Transmembrane signaling rceptor activity, carbohydrate binding
*AKR*1*C*3	<0.001	0.63	Retinal dehydrogenase activity, NADP+1-oxidoreductase activity, aldo-keto reductase (NADP) activity, retinol dehydrogenase activity
*KLRF*1	<0.001	0.62	Transmembrane signaling receptor activity, protein binding, carbohydrate binding, MHC class Ⅰ receptor activity
*KLRB*1	<0.001	0.62	Transmembrane signaling receptor activity, protein binding, carbohydrate binding
*KRT*86	<0.001	0.62	Protein binding
*LEPROTL*1	<0.001	0.61	Protein binding, identical protein binding
FCGBP	<0.001	0.60	Protein binding
*ADAMTS*10	0.01	0.52	Metalloendopeptidase activity, protein binding, peptidase activity, metallopeptidase activity
IGJ	0.04	0.52	Single-stranded DNA binding, antigen binding, IgA binding, protein binding, phosphatidylcholine binding

表 3 患者治疗前和对照组比较、患者治疗后和治疗前比较外周血中的差异表达基因

Table 3 DEGs findings of comparison between peripheral blood of patients before treatment and that of the controls, and DEGs findings of comparison between the peripheral blood of patients after treatment with that of patients before treatment

Gene symbol	Description	Before treating vs. control		After treating vs. before teating	
		P	Fold change	*P*	Fold change
TLR5	Toll-like receptor 5	<0.001	1.55	0.03	0.67
IL1RN	Interleukin 1 receptor antagonist	0.01	1.66	0.03	0.64
SLC8A1	Solute carrier family 8 member A1	<0.001	1.48	0.04	0.63
RBM20	RNA binding motif protein 20	>0.05	–	0.03	0.62
GPER1	G protein-coupled estrogen receptor 1	>0.05	–	0.00	0.62
IL27	Interleukin 27	>0.05	–	0.01	0.59
SOCS1	Suppressor of cytokine signalling 1	>0.05	–	0.02	0.56
GRTP1-AS1	GRTP1 antisense RNA 1	>0.05	–	0.04	0.48

153 differentially expressed genes were identified in the comparison of peripheral blood of the patients before treatment and that of the control group. 8 differentially expressed genes were identified in the comparison of peripheral blood of the patients before treatment and that after treatment. The intersection of the two groups of differentially expressed genes included *TLR5*, *IL1RN* and *SLC8A1*.

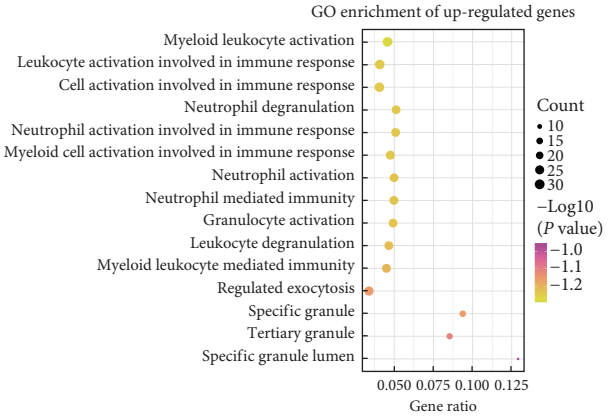

图 1 腰椎间盘脱出患者治疗前和健康对照者外周血样本比较，上调的差异基因富集在以上条目中（*P*值最小的15个条目）

Fig 1 In the comparison of the peripheral blood samples of patients with LDE and those healthy controls, the up-regulated genes were enriched in the above items (15 items with the smallest *P* value)

TLR5(中心度17)等。结合富集分析显示：*TLR4*显著富集到图3中的髓样白细胞活化、白细胞活化参与免疫反应和细胞活化等条目以及对其他生物的防御反应和对细菌的防御反应等条目，*MMP9*显著富集到图2中除特定颗粒和特定颗粒内腔外的全部条目，*MPO*显著富集到图3中除特定颗粒、三级颗粒和特定颗粒内腔外的全部条目，*CAMP*显著富集到图2中的所有条目，*RETN*显著富集到图3中除三级颗粒外的全部条目，*TLR5*显著富集到对其他生物的防御反应和对细菌的防御反应等条目。

MCODE插件筛选出3个关键子网络，这些子网络中包含的中心度最高的DEGs包括*TLR4*、*MMP9*、*MPO*、*CAMP*和*RETN*。综合PPI网络的中心度和关键子网络分析，*TLR4*、*MMP9*、*MPO*、*CAMP*、*RETN*和*TLR5*在患者外周血基因表达失调中起主要作用。

2.5 qRT-PCR结果

我们用qRT-PCR检测了患者治疗前的外周血液样本和健康对照者外周血样本中*TLR4*、*MMP9*、*MPO*、

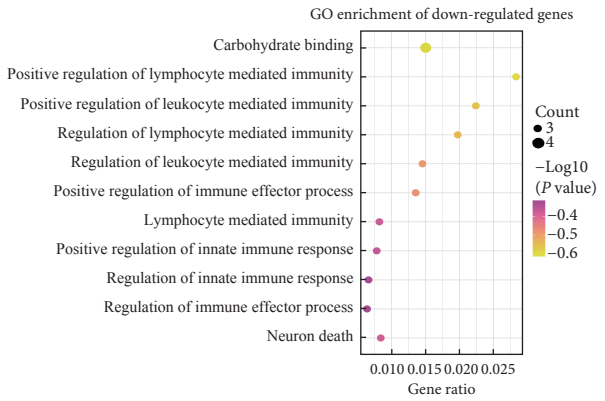

图 2 腰椎间盘脱出患者治疗前和健康对照者外周血样本比较，下调的差异表达基因富集在以上11个条目中

Fig 2 In the comparison of the peripheral blood samples of patients with LDE and those of healthy controls, the up-regulated genes were enriched in the above items

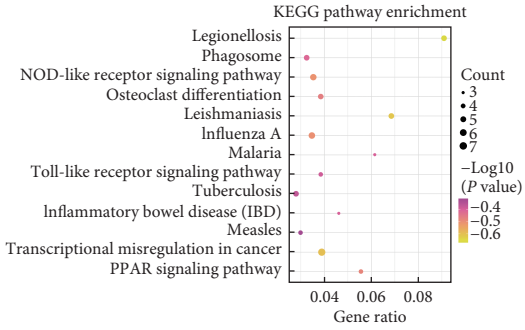

图 3 差异表达基因KEGG通路富集分析结果

Fig 3 The results of KEGG pathway enrichment analysis for differentially expressed genes (DEGs)

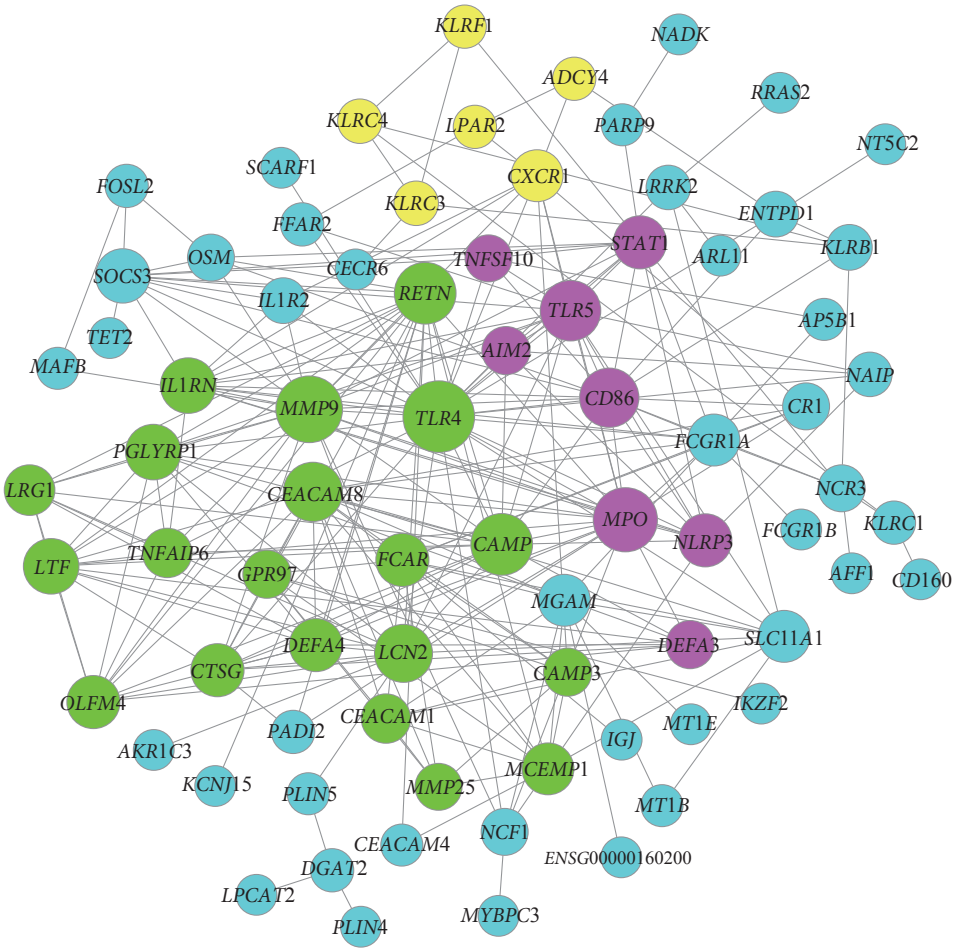

图 4 PPI网络

Fig 4 The PPI network

The PPI network consisted of 77 nodes and 255 edges. The size of the node represented the centrality degree of the node. The three key sub-networks selected by the MEODE plug-in were displayed in green, purple, and yellow in the PPI network.

表 4 PPI网络中具有最高中心度数的15个基因及其是否被包含在MCODE子网络中

Table 4 The 15 genes with the highest centrality degree in the PPI network and whether they were included in the MCODE sub-network

Gene symbol	Centrality degree	MCODE_Cluster
TLR4	26	Cluster 1
MMP9	22	Cluster 2
MPO	20	Cluster 2
CAMP	18	Cluster 2
RETN	18	Cluster 1
TLR5	17	Unclustered
CEACAM8	16	Cluster 1
CD86	16	Cluster 2
LCN2	15	Cluster 1
IL1RN	13	Cluster 1
PGLYRP1	13	Cluster 1
LTF	13	Cluster 1
SOCS3	12	Unclustered
CTSG	11	Cluster 1
STAT1	11	Cluster 2

CAMP、*RETN*、*TLR5*、*IL1RN*和*SLC8A1*的表达量，发现这些基因在患者治疗前的外周血液样本中表达量明显高于在健康对照者外周血样本的表达量(图5)。qRT-PCR结果与基因芯片结果一致，提示*TLR4*、*MMP9*、*MPO*、*CAMP*、*RETN*和*TLR5*是*LDE*患者外周血基因表达失调的关键基因。此外，我们还用qRT-PCR检测了患者治疗前和治疗后的外周血液样本中*TLR5*、*IL1RN*和*SLC8A1*的表达量，发现和非手术治疗前比较这些基因的表达量在非手术治疗后明显降低(图5)。

3　讨论

现有研究报道的腰椎间盘突出症外周血生物标志物大多同免疫和炎症反应相关[9-16]，这和本研究结果类似。但是这些外周血生物标志物和本研究发现的关键基因之间没有重叠，可能是因为本研究所纳入的病理类型、人种、检测方法等和既往的研究不同所导致。

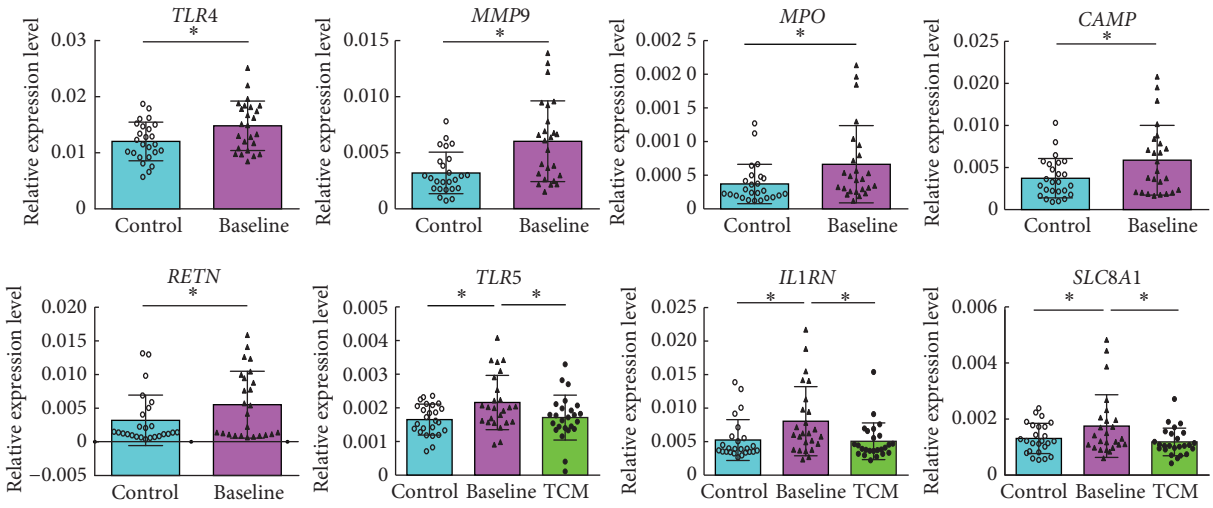

图 5 腰椎间盘脱出患者治疗前后外周血*TLR4*、*MMP9*、*MPO*、*CAMP*、*RETN*、*TLR5*、*IL1RN*和*SLC8A1*基因表达结果

Fig 5 The expression levels of *TLR4*, *MMP9*, *MPO*, *CAMP*, *RETN*, *TLR5*, *IL1RN* and *SLC8A1* in the peripheral blood of LDE patients before and after treatment

Control: Healthy volunteers; Baseline: Patients with LDE before nonoperative treatment; TCM: Patients with LDE after traditional Chinese medicine nonoperative treatment. n=25, * P<0.05.

本研究发现*TLR4*、*MMP9*、*MPO*、*CAMP*、*RETN*和*TLR5*在PPI网络中具有较高的中心度数，表明这些基因是PPI网络的关键基因，其中*TLR4*具有最高的中心度数，是核心关键基因。在所有Toll样受体中，*TLR4*因在炎症反应中发挥了积极的作用而被广泛研究[17]。动物模型证实椎间盘内*TLR4*的表达和激活可诱发炎症反应，引起*TNF-α*、*IL-1β*、*IL-6*和一氧化氮表达升高[18]。除此之外*TLR4*还参与先天性神经免疫和神经病理介导的神经病理性疼痛[19]。阻断椎间盘内TLR4可以减轻炎症反应并逆转疼痛相关的神经重塑，提示*TLR4*可能是治疗椎间盘相关炎症和神经病理性疼痛的潜在目标基因[20]。椎间盘富含胶原并有*MMP9*表达，而*MMP9*的主要作用之一是降解胶原[21]，*MMP9*在不同类型的突出椎间盘中表达不同，其在膨出椎间盘、突出椎间盘和游离椎间盘内的表达量依次递增[22]。此外*MMP9*还通过目前尚不明确的机制参与炎症反应的激活和阻断[23]。局部的*MMP9*可以促进白细胞从外周血向组织迁移，产生趋化梯度，从而在神经炎性反应中发挥作用[24]。*MPO*主要由血液循环中的中性粒细胞产生，与炎症和神经系统退变疾病相关，*MPO*产生次氯酸-鞘磷脂酶，而次氯酸-鞘磷脂酶的释放是活性氧的来源，*MPO*由此间接参与活性氧介导的炎性损伤[25]。*MPO*在中风患者血浆中[26]、阿尔茨海默病[27]和帕金森患者[28]脑组织中、多发性硬化斑块中[29]表达均升高，在神经病理性疼痛中，脊髓和坐骨神经内的*MPO*连同丙二醛、一氧化氮等同步显著升高[30]，尽管*MPO*在这些神经系统疾病的作用仍未完全明确，但可以表明*MPO*与神经系统的退变和损伤之间存在密切的联系。*RETN*是一种具有促炎特性的脂肪因子，它可以上调人外周血单核细胞中*TNF-α*和*IL-6*的表达以及巨噬细胞中*TNF-α*和*IL-12*的表达[31-32]。大量研究显示在很多炎性条件下，*RETN*水平和血浆中的*TNF-α*受体2、*IL-6*、*ICAM-1*、脂蛋白相关*PLA2*以及C反应蛋白等炎症标志物相关联[33-34]。*CAMP*由急性炎症区域内的活化中性粒细胞和单核细胞所分泌，与免疫复合物沉积性小血管炎和新月体肾炎关系密切[35]，是否与突出间盘的血管化相关需要进一步的研究。

本研究还发现在非手术治疗前后，随疼痛的缓解和症状的恢复，*TLR5*、*IL1RN*和*SLC8A1*在患者外周血中表达由高到低，表明三者在疾病的恢复中发挥了一定作用。*TLR5*被发现与*TLR4*一样属于高迁移率族蛋白(high mobility group box 1, HMGB1)受体，通过活化NF-κB信号通道导致促炎因子生成及疼痛的增加[36]，动物实验发现敲除或阻断*TLR5*可以减轻疼痛[37]，本研究*TLR5*水平降低可能代表着NF-κB信号通道的阻断而减轻炎性反应。一些学者研究了*IL1RN*基因的多态性和椎间盘退变性疾病的关系[38-39]，对取出的突出间盘细胞培养时发现，这些细胞能自发地分泌*IL1RN*[40]，这可能提示随症状的缓解，突出间盘细胞分泌*IL1RN*的能力下降，从而表现出*IL1RN*水平的降低。*SLC8A1*，作为一种Na^+/Ca^{2+}交换器，在炎症条件下，能导致Na^+内流的加速和Ca^{2+}的丢失，是炎症的扩大器[41]，*SLC8A1*水平下调，可能意味着炎症水平的控制。

LDE是在椎间盘退变基础发生的纤维环破裂、髓核脱出，椎间盘退变是伴随年龄增长的一种生理现象，但是

目前对椎间盘从什么年龄开始发生退变尚无定论, 如果以和患者年龄匹配的人群为对照, 对照组则可能存在明显椎间盘退变或影像检查不能显示的早期椎间盘退变, 由此可导致研究结果的偏倚。因年轻人椎间盘未开始退变或椎间盘退变处于起始阶段, 本研究纳入年轻人为对照大大降低了这类偏倚, 但是也使得研究结果受到年龄的影响, 这是本研究的局限所在。

综上所述, 本研究发现LDE患者外周血基因表达特征为免疫和炎症反应相关基因表达失调, *TLR*4、*MMP*9、*MPO*、*CAMP*、*RETN*和*TLR*5是这些基因表达失调的关键基因, 非手术治疗疗效的获得可能和患者外周血中过度表达的*TLR*5、*IL*1*RN*和*SLC*8*A*1下调相关。炎症反应是一个续贯过程, 本次研究的时间较短, 但也有不少有价值的发现, 椎间盘突出后涉及大量的炎症因子, 这些炎症因子又与神经病理性疼痛之间存在着密切的联系。我们下一步的目标是对椎间盘突出全程的基因监控, 包括急性症状期、缓解期及突出间盘吸收期, 找出其表达的规律, 聚焦导致神经病理性疼痛的关键基因, 寻找能够用于预测腰椎间盘突出发生重吸收的生物标志物, 为腰椎间盘突出的临床决策提供高质量的证据。

*　　*　　*

利益冲突　所有作者均声明不存在利益冲突

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