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miR-4463 在下肢动脉硬化闭塞症中的表达及意义

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[关键词] miR-4463； 下肢动脉硬化闭塞症； miRNA 芯片方法

[摘 要] **目的** 观察 microRNA-4463(miR-4463)在下肢动脉硬化闭塞症(ASO)患者血浆和组织中的表达变化，推测其可能意义。**方法** 采用 miRNA 芯片方法筛选 ASO 患者和对照人群血浆中差异表达 miRNA，Real time PCR 技术验证 ASO 患者和对照人群中 miR-4463 的表达水平，并进行临床分期比较。靶基因预测软件分析 miR-4463 靶基因，Gene Ontology 和 KEGG 数据库分析靶基因的功能和信号通路。**结果** 与对照组相比，ASO 患者血浆中有 51 个 miRNA 变化超过 1.5 倍($P<0.05$)。miR-4463 在 ASO 患者血浆和病变血管内膜组织中表达均显著降低，并随 Fontaine 分期呈逐渐下降趋势。生物信息学分析发现 miR-4463 靶基因与细胞迁移、脂质代谢、内吞作用等相关。**结论** miR-4463 参与了 ASO 的病程，其表达下降可能提示 ASO 的发生。

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The expression of miR-4463 in patients with arteriosclerosis obliterans

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[**KEY WORDS**] miR-4463; Arteriosclerosis obliterans; miRNA microarray method

[**ABSTRACT**] **Aim** To observe the expression of miR-4463 in the plasma and tissues of patients with arteriosclerosis obliterans of lower limbs (ASO). **Methods** miRNA microarray method was used to screen the differentially expressed miRNA in the plasma of three ASO patients and three controls. Real time PCR technique was carried out to verify the expression level of miR-4463 in the plasma and tissues of 50 patients with ASO and 50 healthy controls, and the relationship of clinical stage with miR-4463 level was analyzed. Target gene prediction software was used to predict target genes of miR-4463, and then the function and signaling pathways of predicted target genes were analyzed by Gene Ontology and KEGG pathway. **Results** Compared with the controls, 51 miRNAs changed in the plasma of patients with ASO more than 1.5 times ($P<0.05$). The expression of miR-4463 in plasma and vascular intimal tissues of ASO patients was significantly decreased, and decreased gradually with Fontaine stage. Bioinformatics analysis revealed that the target genes of miR-4463 were related to cell polarity, migration, lipid metabolism and endocytosis. **Conclusion** miR-4463 is involved in the pathogenesis of ASO, and decreased miR-4463 level may indicate the occurrence of ASO.

下肢动脉硬化闭塞症(arteriosclerosis obliterans of lower limbs，ASO)是常见的外周动脉闭塞性疾病，主要累及腹主动脉、双髂动脉、双股动脉、双胭动脉及其远端 3 个分支。其主要危害是增加心脑血管事件的发生风险，也是导致中老年人下肢截肢的主要原因之一，严重影响患者的生活质量[1]。患者在疾病早期阶段缺乏明确的临床症状，常常因此延误治疗而被迫截肢，因此，寻找能在早期阶段提示 ASO 的分子标志具有重要意义。microRNA(miRNA)是近年发现的一类内源性、非编码小分子 RNA，通过在转录后水平抑制靶基因的翻译调控细胞的生长、增殖、迁移及凋亡[2]。研究发现循环 miRNA 的改变与肿瘤、糖尿病和心血管疾病等多种疾病的发生相关，并在发病早期即有改变[3-5]。本研究用 miRNA 芯片技术筛选了 3

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例 ASO 患者和 3 例对照人群血浆 miRNA 的表达谱差异,并通过 Real time PCR 进行验证,旨在寻找 ASO 早期即有改变的特异性 miRNA。

1 资料和方法

1.1 研究对象

选取 2013 年 10 月至 2016 年 8 月就诊于本院确诊为下肢动脉硬化闭塞症的患者(ASO 组)50 例,健康对照人群(对照组)50 例,两组人群性别、年龄无统计学差异,一般资料见表 1。按照 Fontaine 分期标准对 ASO 患者进行分期,由 3 名血管外科医师共同完成,其中 Fontaine Ⅰ 期 6 例,Fontaine Ⅱ 期 12 例,Fontaine Ⅲ 期 16 例,Fontaine Ⅳ 期 16 例。患者均排除冠心病、脑卒中、肝肾功能衰竭、肿瘤。采集受试者 EDTA 抗凝血 3 mL,3000 g 离心 10 min,收集血浆,每管 500 μL 分装保存于-80℃。病变血管组织标本取自于截肢患者,共 20 例;正常对照血管取自车祸截肢患者,共 5 例。截肢后,迅速解剖出股动脉,生理盐水冲洗掉血液,剥离血管周围组织,RNA 保护液 4℃放置过夜后于-80℃保存。受试者均获得知情同意书,所有标本取材均通过西南医科大学附属医院伦理委员会批准。

表 1. 研究人群一般资料

Table 1. General data of study population

一般资料	ASO 组(n=50)	对照组(n=50)
年龄(岁)	75.72+10.52	73.67+7.92
男/女 (例)	27/23	26/24
吸烟(例)	10	8
饮酒(例)	9	6
高血压(例)	17	0
糖尿病(例)	13	0

1.2 RNA 提取

取 400 μL 血浆按照试剂盒说明书(miRNeasy Serum/Plasma Kit, QIAGEN)提取总 RNA。加入 7 μL 1.6×10^8 拷贝 Spike-In (Caenorhabditis elegans miRNA 39, cel-miR-39)作为外参用以评估血浆 RNA 提取过程。血管内膜组织 RNA 提取按试剂盒说明书(miRNeasy Mini Kit , QIAGEN)进行。

1.3 miRNA 芯片杂交与分析

用 miRCURY™ Hy3™/Hy5™ Power labeling kit 将提取的血浆总 RNA 进行标记,杂交至 miRCURY™ LNA 芯片(v.18.0),然后以 Axon GenePix 4000B microarray scanner 扫描数据,GenePix Pro 6.0 软件进行分析结果。

1.4 逆转录

取上述 miRNA 溶液 6 μL 参照试剂盒说明书(miScript Ⅱ RT Kit, QIAGEN)进行逆转录。轻柔混匀后瞬时离心,于 PCR 仪中进行反转录,反应条件为 37℃ 60 min,然后 95℃ 5 min。cDNA 溶液保存于-20℃。

1.5 Real time PCR 检测 miR-4463 表达水平

在 10 μL cDNA 溶液中加入 100 μL ddH_2O,充分混匀后进行荧光定量 PCR。反应体系为 25 μL,包括 12.5 μL SYBR Green Master Mix(QIAGEN),2.5 μL 10×miRNA 特异性引物(QIAGEN),2.5 μL 10×通用引物,2 μL 稀释的 cDNA,无 RNase ddH_2O 补足至 25 μL。于 ABI StepOne Plus 荧光定量 PCR 仪进行检测,每个样本重复 3 次。PCR 的反应条件为 95℃ 15 min,然后 95℃ 15 s,55℃ 30 s,70℃ 30 s,共 40 个循环。以 cel-miR-39 和 U6 分别作为血浆和组织的内参,$2^{-\Delta\Delta Ct}$ 法计算相对表达倍数。

1.6 生物信息学分析

以 miRNA 在线靶基因预测软件 TargetScan 数据库(http://www.targetscan.org/)和 miRDB 数据库(http://www.mirdb.org/)预测 miR-4463 靶基因,并对靶基因进行 Gene Ontology 和 Kyoto Encyclopedia of Genes and Genomes(KEGG)pathway 分析(https://david.ncifcrf.gov/)。

1.7 统计学分析

使用 SPSS 19.0 软件分析实验数据,计量资料以 $\bar{x}\pm s$ 表示,两组间数据比较采用独立样本 t 检验,多组间比较采用单因素方差分析,$P<0.05$ 表示差异具有统计学意义。

2 结 果

2.1 血浆 miRNA 表达谱分析

选取 3 例单纯 ASO 患者和 3 例性别年龄匹配的对照者进行血浆 miRNA 芯片杂交,结果表明,与对照相比,有 51 个 miRNA 的变化超过 1.5 倍($P<0.05$),其中 8 个 miRNA 表达上调,43 个 miRNA 表达下调(图 1)。

2.2 miR-4463 在血浆和血管内膜组织中的表达

采用 Real time PCR 检测 50 例 ASO 患者和 50 例对照人群血浆 miR-4463 的表达水平,结果表明,miR-4463 在 ASO 患者血浆表达下降(图 2A)。随后在血管内膜组织中进行 miR-4463 表达水平检测,

结果发现病变血管内膜中 miR-4463 表达降低(图 2B),差异有统计学意义。

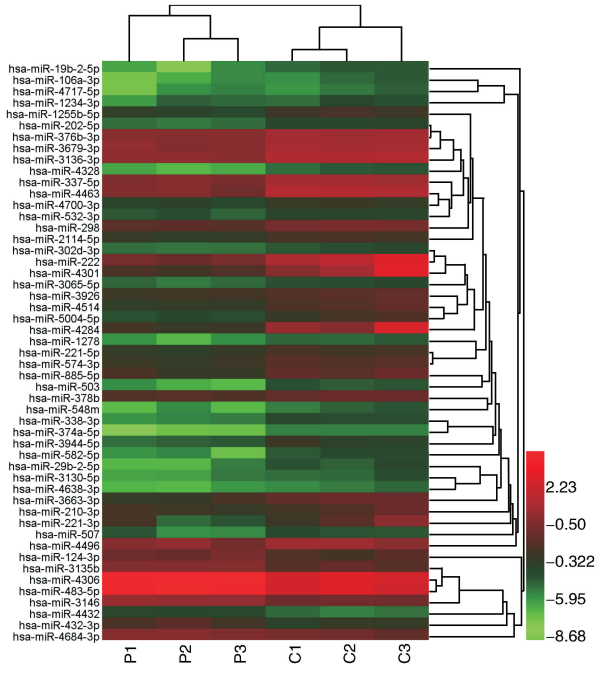

图 1. ASO 患者血浆中差异表达 miRNA P1、P2、P3 代表 ASO 患者;C1、C2、C3 代表对照人群。

Figure 1. Altered miRNA expression in plasma of ASO patients

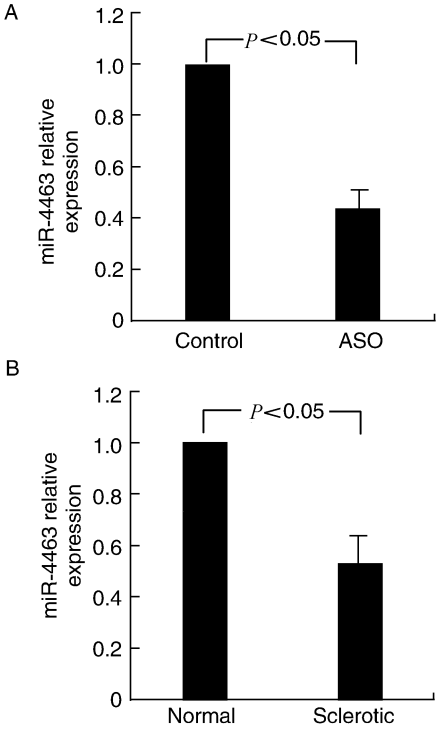

图 2. qPCR 检测血浆(A)和血管内膜(B)中 miR-4463 表达水平

Figure 2. miR-4463 expression in plasma (A) and intima (B) detected by qPCR

2.3 miR-4463 的表达水平与 ASO 分期的关系

为了检测 ASO 患者中 miR-4463 的变化是否与临床分期相关,将 50 例 ASO 患者按 Fontaine 分期法分为 4 期,并比较不同分期患者中 miR-4463 的表达水平。结果表明,miR-4463 的表达水平在Ⅰ期显著下降,并随分期呈逐渐下降趋势(图 3)。

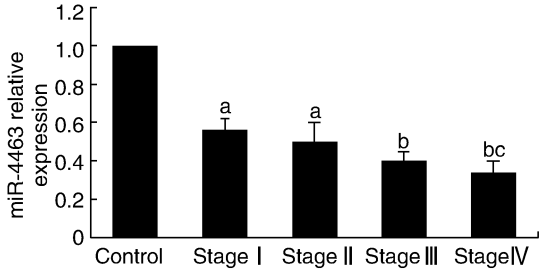

图 3. miR-4463 表达水平与 ASO 患者 Fontaine 分期的关系

a 为 $P<0.05$,b 为 $P<0.01$,与对照组比较;c 为 $P<0.05$,与 FontaineⅠ期比较。

Figure 3. The correlation of miR-4463 expression with Fontaine stages in ASO patients

2.4 miR-4463 靶基因预测分析

分别以 TargetScan 和 miRDB 数据库预测 miR-4463 靶基因后,筛选其中在 mRNA 3′UTR 区至少含有 7 个结合位点的靶基因,取两数据库结果的交集,筛选文献报道可能与 ASO 相关的靶基因进行 Gene Ontology 分析。如图 4 所示,这些预测靶基因包括 BCL2L11、AMOT、CLDN1、FGF1、PDGFRL 等,它们与细胞极性和迁移、细胞的紧密连接、脂质代谢、内吞作用、血管平滑肌的收缩等功能相关。KEGG pathway 分析发现他们可能参与了胰岛素信号通路、PI3K-Akt 信号通路、AMPK 信号通路、mTOR 信号通路等(图 5)。

3 讨 论

ASO 为动脉硬化造成的下肢供血动脉内膜增厚、管腔狭窄或闭塞,临床表现为下肢间歇性跛行、皮温降低、疼痛、甚至溃疡或坏死。ASO 的临床诊断辅助检查如血管造影、多普勒超声、核磁共振、多层螺旋 CT、踝臂指数(ankle brachial index, ABI)测定等适用于 ASO 已经发生后病变程度的鉴定;实验室检查主要有血脂、血糖、肾功能等,这些结果很难在 ASO 早期指示其发生,常常延误患者治疗。因此,在前人基础上进一步筛选鉴定易于获得的 ASO 早期标志分子迫在眉睫。

miRNA 是一类大小为 18~22 bp 的内源性非编

BCL2L11	BCL2-like 11 (apoptosis facilitator)	Related Genes	Homo sapiens
GOTERM_MF_FAT	microtubule binding, cytoskeletal protein binding, tubulin binding,		
IQSEC2	IQ motif and Sec7 domain 2	Related Genes	Homo sapiens
GOTERM_MF_FAT	small GTPase regulator activity, guanyl-nucleotide exchange factor activity, ARF guanyl-nucleotide exchange factor activity, GTPase regulator activity, nucleoside-triphosphatase regulator activity,		
AMOT	angiomotin	Related Genes	Homo sapiens
GOTERM_MF_FAT	angiostatin binding,		
ARRB1	arrestin, beta 1	Related Genes	Homo sapiens
GOTERM_MF_FAT	enzyme inhibitor activity,		
CLDN1	claudin 1	Related Genes	Homo sapiens
GOTERM_MF_FAT	structural molecule activity, protein domain specific binding, PDZ domain binding, identical protein binding,		
CLDN19	claudin 19	Related Genes	Homo sapiens
GOTERM_MF_FAT	magnesium ion binding, structural molecule activity, identical protein binding, ion binding, cation binding, metal ion binding,		
CYTH3	cytohesin 3	Related Genes	Homo sapiens
GOTERM_MF_FAT	small GTPase regulator activity, guanyl-nucleotide exchange factor activity, ARF guanyl-nucleotide exchange factor activity, phospholipid binding, phosphatidylinositol binding, phosphatidylinositol-3,4,5-trisphosphate binding, inositol-1,4,5-trisphosphate receptor activity, lipid binding, GTPase regulator activity, phosphoinositide binding, nucleoside-triphosphatase regulator activity,		
FGF1	fibroblast growth factor 1 (acidic)	Related Genes	Homo sapiens
GOTERM_MF_FAT	pattern binding, glycosaminoglycan binding, growth factor activity, heparin binding, carbohydrate binding, polysaccharide binding,		
FGF2	fibroblast growth factor 2 (basic)	Related Genes	Homo sapiens
GOTERM_MF_FAT	pattern binding, ion channel activity, voltage-gated ion channel activity, voltage-gated calcium channel activity, cation channel activity, calcium channel activity, glycosaminoglycan binding, growth factor activity, heparin binding, channel activity, passive transmembrane transporter activity, voltage-gated channel activity, gated channel activity, substrate specific channel activity, voltage-gated cation channel activity, carbohydrate binding, polysaccharide binding, metal ion transmembrane transporter activity,		
FGF5	fibroblast growth factor 5	Related Genes	Homo sapiens
GOTERM_MF_FAT	growth factor activity,		
FZD4	frizzled homolog 4 (Drosophila)	Related Genes	Homo sapiens
GOTERM_MF_FAT	Wnt-protein binding, Wnt receptor activity,		
HK2	hexokinase 2 pseudogene; hexokinase 2	Related Genes	Homo sapiens
GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, hexokinase activity, ATP binding, sugar binding, glucose binding, purine nucleotide binding, carbohydrate kinase activity, carbohydrate binding, adenyl nucleotide binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding, monosaccharide binding,		
INSR	insulin receptor	Related Genes	Homo sapiens
GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, protein kinase activity, protein tyrosine kinase activity, transmembrane receptor protein tyrosine kinase activity, insulin receptor activity, insulin-like growth factor receptor binding, insulin-like growth factor binding, ATP binding, GTP binding, peptide hormone binding, purine nucleotide binding, guanyl nucleotide binding, growth factor binding, enzyme binding, kinase binding, protein kinase binding, phosphatase binding, protein phosphatase binding, protein domain specific binding, adenyl nucleotide binding, lipoic acid binding, carboxylic acid binding, insulin-like growth factor I binding, insulin-like growth factor II binding, protein complex binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding, guanyl ribonucleotide binding, SH2 domain binding, peptide binding, hormone binding, 3-phosphoinositide-dependent protein kinase binding, phosphoinositide 3-kinase binding, insulin binding, insulin receptor substrate binding, cofactor binding, coenzyme binding, PTB domain binding,		
IDE	insulin-degrading enzyme	Related Genes	Homo sapiens
GOTERM_MF_FAT	nucleotide binding, nucleoside binding, purine nucleoside binding, endopeptidase activity, metalloendopeptidase activity, ATP binding, peptidase activity, metallopeptidase activity, zinc ion binding, ATPase activity, peptide hormone binding, purine nucleotide binding, adenyl nucleotide binding, beta-endorphin binding, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding, neurotransmitter binding, peptide binding, hormone binding, identical protein binding, protein homodimerization activity, ion binding, cation binding, insulin binding, metal ion binding, transition metal ion binding, protein dimerization activity, peptidase activity, acting on L-amino acid peptides,		
PDE3B	phosphodiesterase 3B, cGMP-inhibited	Related Genes	Homo sapiens
GOTERM_MF_FAT	cyclic-nucleotide phosphodiesterase activity, 3',5'-cyclic-nucleotide phosphodiesterase activity, 3',5'-cyclic-AMP phosphodiesterase activity, cGMP-inhibited cyclic-nucleotide phosphodiesterase activity, phosphoric diester hydrolase activity, enzyme binding, kinase binding, protein kinase binding, protein kinase B binding,		
PDGFRL	platelet-derived growth factor receptor-like	Related Genes	Homo sapiens
GOTERM_MF_FAT	protein kinase activity, protein tyrosine kinase activity, transmembrane receptor protein tyrosine kinase activity, platelet activating factor receptor activity, platelet-derived growth factor receptor activity,		
PTGER3	prostaglandin E receptor 3 (subtype EP3)	Related Genes	Homo sapiens
GOTERM_MF_FAT	ligand-dependent nuclear receptor activity, icosanoid receptor activity, prostanoid receptor activity, prostaglandin receptor activity, prostaglandin E receptor activity,		
PRKAA2	protein kinase, AMP-activated, alpha 2 catalytic subunit	Related Genes	Homo sapiens
GOTERM_MF_FAT	nucleotide binding, magnesium ion binding, nucleoside binding, purine nucleoside binding, protein kinase activity, protein serine/threonine kinase activity, AMP-activated protein kinase activity, ATP binding, purine nucleotide binding, adenyl nucleotide binding, protein binding, bridging, ribonucleotide binding, purine ribonucleotide binding, adenyl ribonucleotide binding, ion binding, cation binding, metal ion binding,		
PRKAB2	protein kinase, AMP-activated, beta 2 non-catalytic subunit	Related Genes	Homo sapiens
GOTERM_MF_FAT	enzyme binding, kinase binding, protein kinase binding,		
PPP1R10	protein phosphatase 1, regulatory (inhibitor) subunit 10	Related Genes	Homo sapiens
GOTERM_MF_FAT	DNA binding, RNA binding, enzyme inhibitor activity, phosphoprotein phosphatase inhibitor activity, protein phosphatase 1 binding, zinc ion binding, phosphatase regulator activity, phosphatase inhibitor activity, protein phosphatase regulator activity, enzyme binding, phosphatase binding, protein phosphatase binding, transcription regulator activity, ion binding, cation binding, metal ion binding, transition metal ion binding,		

图 4. miR-4463 预测靶基因 Gene Ontology 分析结果

Figure 4. Gene ontology analysis of miR-4463 predicted target genes

IQSEC2	IQ motif and Sec7 domain 2	Related Genes	Homo sapiens
KEGG_PATHWAY	Endocytosis,		
ARRB1	arrestin, beta 1	Related Genes	Homo sapiens
KEGG_PATHWAY	MAPK signaling pathway, Chemokine signaling pathway, Endocytosis,		
CLDN1	claudin 1	Related Genes	Homo sapiens
KEGG_PATHWAY	Cell adhesion molecules (CAMs), Tight junction, Leukocyte transendothelial migration, Pathogenic Escherichia coli infection,		
CLDN19	claudin 19	Related Genes	Homo sapiens
KEGG_PATHWAY	Cell adhesion molecules (CAMs), Tight junction, Leukocyte transendothelial migration,		
FGF1	fibroblast growth factor 1 (acidic)	Related Genes	Homo sapiens
KEGG_PATHWAY	MAPK signaling pathway, Regulation of actin cytoskeleton, Pathways in cancer, Melanoma,		
FGF2	fibroblast growth factor 2 (basic)	Related Genes	Homo sapiens
KEGG_PATHWAY	MAPK signaling pathway, Regulation of actin cytoskeleton, Pathways in cancer, Melanoma,		
FGF5	fibroblast growth factor 5	Related Genes	Homo sapiens
KEGG_PATHWAY	MAPK signaling pathway, Regulation of actin cytoskeleton, Pathways in cancer, Melanoma,		
FZD4	frizzled homolog 4 (Drosophila)	Related Genes	Homo sapiens
KEGG_PATHWAY	Wnt signaling pathway, Melanogenesis, Pathways in cancer, Colorectal cancer, Basal cell carcinoma,		
HK2	hexokinase 2 pseudogene; hexokinase 2	Related Genes	Homo sapiens
KEGG_PATHWAY	Glycolysis / Gluconeogenesis, Fructose and mannose metabolism, Galactose metabolism, Starch and sucrose metabolism, Amino sugar and nucleotide sugar metabolism, Insulin signaling pathway, Type II diabetes mellitus,		
INSR	insulin receptor	Related Genes	Homo sapiens
KEGG_PATHWAY	Adherens junction, Insulin signaling pathway, Type II diabetes mellitus, Aldosterone-regulated sodium reabsorption,		
IDE	insulin-degrading enzyme	Related Genes	Homo sapiens
KEGG_PATHWAY	Alzheimer's disease,		
PDE3B	phosphodiesterase 3B, cGMP-inhibited	Related Genes	Homo sapiens
KEGG_PATHWAY	Purine metabolism, Insulin signaling pathway, Progesterone-mediated oocyte maturation,		
PTGER3	prostaglandin E receptor 3 (subtype EP3)	Related Genes	Homo sapiens
KEGG_PATHWAY	Calcium signaling pathway, Neuroactive ligand-receptor interaction,		
PRKAA2	protein kinase, AMP-activated, alpha 2 catalytic subunit	Related Genes	Homo sapiens
KEGG_PATHWAY	Regulation of autophagy, mTOR signaling pathway, Insulin signaling pathway, Adipocytokine signaling pathway, Hypertrophic cardiomyopathy (HCM),		
PRKAB2	protein kinase, AMP-activated, beta 2 non-catalytic subunit	Related Genes	Homo sapiens
KEGG_PATHWAY	Insulin signaling pathway, Adipocytokine signaling pathway, Hypertrophic cardiomyopathy (HCM),		

图 5. miR-4463 预测靶基因 KEGG pathway 分析结果

Figure 5. KEGG pathway analysis of miR-4463 predicted target genes

码小 RNA 分子,参与心血管系统疾病的调控,包括动脉硬化、血管新生、内膜增殖、血管再狭窄等病理生理过程[3-4]。miR-21 是血管平滑肌细胞增殖、迁移和凋亡的重要调节因子[6],miR-155、miR-221、miR-222 和 miR-126 是血管炎性反应的关键调节因子[7-9],miR-210、miR-126、Let-7 具有促进血管新生的功能[10-11]。研究表明,miRNA 的表达具有严格的时空特异性和组织特异性,病变组织中的 miRNA 会释放到循环血中引起循环 miRNA 表达水平改变[12]。循环 miRNA 因其易于通过微创手段获得,又具有高度的敏感性和特异性,甚至在疾病早期即发生明显改变,因此,循环 miRNA 是潜在的疾病诊断标志物。目前,循环 miRNA 作为多种疾病的标志物已相继报道。miR-130a、miR-27b、miR-210 在血管硬化组织和血清中均发生明显改变并有望成为诊断标志物[13];血浆 miR-25-3p 和 let-7d-5p 可作为潜在标志物分辨急性和稳定型特发性肺纤维化[14],这些结果都提示 miRNA 具有较好的临床诊断价值。

Wang 等[15]通过芯片筛选了 ASO 病变血管内膜中差异表达 miRNA,发现其中 12 个上调 2 倍以上,33 个下调超过 1 倍。经过分析发现我们的血浆芯片结果与其组织芯片结果有部分相同,说明部分血浆 miRNA 的变化可反映血管组织的病变。分析发现 ASO 患者血浆和组织筛选出的 miRNA 中下调的 miRNA 占多数,这可能是因为在 ASO 患者中,由于动脉狭窄严重导致动脉壁长期处于缺血状态,这种热缺血导致 RNA 严重降解[16],因此在血管组织中和血浆中检测到多个 miRNA 表达下降。

本研究首先通过 ASO 患者和正常对照的血浆 miRNA 芯片筛选可能指示 ASO 病变的 miRNA,并通过 qPCR 在 ASO 患者血浆和血管内膜组织中进行验证,以期为 ASO 的早期诊断提供血浆标志物。我们通过 Real time PCR 验证发现 miR-4463 在病变血管内膜和血浆中均显著下降,其表达水平在 Fontaine Ⅰ期即明显降低,并随着 ASO 的严重程度表达逐渐下降,提示 miR-4463 在 ASO 的发生发展过程中扮演着重要角色,下降的 miR-4463 水平可能提示 ASO 的发生。研究发现 miR-4463 表达水平在其他疾病中也有改变,Ding 等[17]发现循环 miR-4463 在多囊卵巢综合征患者中显著上升,Lu 等[18]发现循环 miR-4463 表达水平在动脉瘤性蛛网膜下腔出血(aneurysmal subarachnoid hemorrhage, SAH)合并继发性脑梗死(delayed cerebral infarction, DCI)患者中下降,而在无 DCI 的 SAH 患者中表达上升。这些结果也证明 miR-4463 的表达水平与疾病的类型密切相关,但是对疾病的诊断还需结合临床表现和其他检测指标。

动脉粥样硬化的形成与内皮细胞损伤、氧化应激增加、血管平滑肌细胞的增殖、迁移与凋亡、炎性细胞的浸润等过程相关[19]。我们通过生物信息学分析发现 miR-4463 的靶基因包括与凋亡相关基因 BCL2L11,与迁移相关基因 AMOT、CLDN1,与细胞内吞作用相关基因 IQSEC2、ARRB1 等。这些结果进一步说明 miR-4463 表达水平的改变与动脉粥样硬化过程密切相关,其机制可能涉及到影响血管平滑肌的迁移、凋亡、细胞对脂质的吞噬等。

综上所述,ASO 患者血浆中多个 miRNA 表达水平发生改变,miR-4463 在 ASO 患者血浆与血管内膜中均表达下降,其血浆表达水平与 Fontaine 分期相关,并在 Fontaine Ⅰ期即显著下调,提示血浆 miR-4463 的下调可作为指示 ASO 发生的潜在标志。本文尚有不足之处,由于正常股动脉难以获取,导致本研究中正常动脉对照较少,将来还需扩大样本进行验证。本研究只进行了血浆 miRNA 表达谱分析,若能同时将血浆与 ASO 患者病变血管进行 miRNA 表达谱分析,可以排除其它可能因素对血浆 miRNA 表达谱的干扰,同时通过比较,削减差异,更能体现血浆中差异表达 miRNA 的诊断价值。后续在血管内皮细胞和血管平滑肌细胞中深入研究 miR-4463 的功能及其靶基因,可揭示 miR-4463 的异常表达在下肢动脉硬化闭塞过程中的作用,为其作为 ASO 的早期诊断指标和治疗靶点提供依据。

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