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·基础研究·

口腔白斑病组织中环状RNA的差异表达谱分析

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【摘要】　目的　探讨环状RNA（circRNA）在口腔白斑（oral leukoplakia，OLK）组织及正常口腔黏膜组织（normal oral mucosal，NOM）中表达谱的差异及临床意义。**方法**　采用高通量测序技术检测6对OLK和NOM组织中差异表达circRNA，利用qRT-PCR验证所筛选的10个circRNA在6对OLK与NOM组织中的表达情况。通过酶耐受实验和sanger测序验证目标circRNA成环情况，对20对OLK与NOM组织中目标circHLA-C进行qRT-PCR验证。分别使用UCSC基因组浏览器对circHLA-C进行可视化分析；利用GO和KEGG富集分析对差异表达circRNA进行功能分析；TargetScan、MiRanda预测目标circRNA下游miRNA、mRNA，并构建竞争性内源RNA（competing endogenous RNA，ceRNA）网络。**结果**　测序数据显示OLK组织中共有366个显著差异表达的circRNA，包括65个上调和301个下调circRNA。经qRT-PCR验证，筛选的10个circRNA中有7个表达结果同测序一致。且经酶耐受和sanger测序验证，确定上调的circHLA-C是具有反式剪接位点的真正的circRNA。相关性分析显示circHLA-C与OLK的异常增生程度呈正相关。ROC曲线分析提示circHLA-C具有诊断OLK的潜在价值，且具有较高的准确性和特异性。**结论**　circRNA在OLK组织中异常表达，上调的circHLA-C表达可能与OLK异常增生程度相关，对OLK诊断具有指导价值。

【关键词】　环状RNA；　口腔白斑病；　癌前病变；　高通量测序；　差异表达谱；　ceRNA；　miRNA；　circHLA-C；　上皮异常增生

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Differential expression profile of circRNAs in oral leukoplakia

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【Abstract】　Objective　To investigate the differences and clinical significance of circRNA expression profiles in oral leukoplakia (OLK) tissues and normal oral mucosal (NOM) tissues. **Methods**　High-throughput sequencing was used to detect differentially expressed circRNAs in 6 pairs of OLK and NOM tissues, and qRT-PCR was used to verify

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the expression of 10 circRNAs screened in 6 pairs of OLK and NOM tissues. The ring formation of circRNA was verified by RNase R digestion and Sanger sequencing, and the target circHLA-C was further verified by qRT-PCR in 20 pairs of OLK and NOM tissues. CircHLA-C was visualized using the UCSC genome browser (genome.ucsc.edu). The function of differentially expressed circRNAs was analyzed by GO and KEGG enrichment analyses. TargetScan and miRanda predicted the downstream miRNAs and mRNAs of the target circRNAs, and a ceRNA network related to the identified circRNAs was constructed in Cytoscape. **Results** Sequencing analysis showed that 366 circRNAs were significantly differentially expressed in OLK tissues, including 65 upregulated and 301 downregulated circRNAs. After qRT-PCR verification, 7 of the 10 screened circRNAs were expressed consistent with the sequencing results. The upregulated circHLA-C was confirmed to be a real circRNA with back-splice junction sites by RNase R digestion and Sanger sequencing. Correlation analysis showed a positive correlation between circHLA-C and the degree of OLK dysplasia. ROC curve analysis suggested that circHLA-C had potential value in diagnosing OLK with high accuracy and specificity. **Conclusion** CircRNA was significantly abnormally expressed in OLK tissues, and the upregulation of circHLA-C may be related to the degree of OLK dysplasia, providing guiding value for the diagnosis of OLK in the future.

【Key words】 circRNA; oral leukoplakia; premalignant lesion; high-throughput sequencing; differential expression profile; competing endogenous RNA; miRNA; circHLA-C; epithelial dysplasia
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口腔白斑(oral leukoplakia,OLK)是一种发生在口腔黏膜上以白色为主、不能擦除的斑片或斑块,临床或病理上不能诊断为其他任何疾病的口腔黏膜潜在恶性疾病。流行病学显示OLK的恶性转化率高达3%～18%,非均质的OLK恶性转化率甚至高达15%～40%[1]。OLK的病因较为复杂,发病机理仍不清楚,目前临床上没有特效治疗方法,因此研究OLK发病机制并确定新的潜在分子标志物具有重要意义。环状RNA(circRNA)是具有共价闭环结构的非编码RNA[2]。有报道表明circRNA在肿瘤、神经系统、心血管疾病、炎症及免疫疾病中发挥重要的作用[3-7]。研究表明circRNA可作为有效的生物标志物和治疗靶点[8-11]。但目前还没有OLK相关circRNA的报道。本研究通过高通量测序描绘OLK和正常口腔黏膜(normal oral mucosal, NOM)组织中circRNA的表达谱,并通过对筛选出的显著差异表达的circRNA进行验证分析,同时构建circRNA-miRNA-mRNA的竞争性内源RNA(competing endogenous RNA,ceRNA)网络,为circRNA在OLK发病机制中的机理研究提供新的研究方向。

1　资料和方法

1.1　研究对象

选取2018年9月至11月于上海第九人民医院口腔黏膜科接受治疗的OLK患者的白斑组织26例,男女比例1∶1。患者的组织样本都通过组织病理学检查确诊为OLK。26例NOM组织来自整复外科及口腔外科拔牙的手术患者。组织纳入标准为不伴有系统性疾病,且3个月内未接受过免疫刺激治疗(包括类固醇治疗),无放疗、化疗史。所有的组织样本取样后都立即放入液氮保存。提供组织样本的患者均于术前签署知情同意书,本研究已通过上海第九人民医院伦理委员会审批[审批编号:(2012)21]。从收集的52例样本中选取6例OLK组织及6例NOM组织进行高通量测序,样本信息见表1,并采用qRT-PCR验证这52例组织中筛选重点circRNA的表达情况。

1.2　主要试剂和仪器

TRIzol裂解液(Life Technologies, 美国);Ribo-Zero rRNA Removal Kits(Illumina, 美国);TruSeq Stranded Total RNA Library Prep Kit(Illumina, 美国);SuperScript Ⅲ逆转录试剂盒(Invitrogen, 美国);qRT-PCR引物(上海云序生物工程技术有限公司);qPCR SYBR Green master mix(上海云序生物科技有限公司);RNaseR(epicentre,美国);RNase inhibitor(Millipore,美国)。NanoDrop ND-1000仪器(Thermo Fisher Scientific,美国);BioAnalyzer 2100仪器(Agilent Technologies,美国);Illumina HiSeq 4000测序平台(上海云序生物科技有限公司);实时荧光定量PCR仪(Thermo Fisher,美国);Gene Amp PCR System 9700(应用生物系统公司,美国)。

表1　高通量测序口腔白斑组织的临床特点

Table 1　The clinical characteristics of patients with oral leukoplakia for sequencing

Number	Age (year)	Sex	Location	Dysplasia degree	Smoking history (No/Yes)	Alcohol history (No/Yes)
1	47	Female	Dorsal of the tongue	Mild	No	No
2	61	Female	Ventral of the tongue	Mild	No	No
3	41	Male	Ventral of the tongue	Mild	No	No
4	51	Female	Ventral of the tongue	Mild-moderate	No	No
5	69	Male	Ventral of the tongue	Mild-moderate	No	Yes
6	52	Female	Buccal	Moderate-severe	No	No

1.3　RNA提取及质控

使用TRIzol试剂盒提取总RNA，使用NanoDrop ND-1000仪测量每个样品的RNA浓度。以吸光光度（OD）$_{260/280}$值作为RNA纯度指标。使用Ribo-Zero rRNA Removal Kits移除总RNA中的rRNA。采用TruSeq Stranded Total RNA Library Prep Kit预处理RNA，构建测序文库。采用BioAnalyzer 2100仪器进行文库质控和定量。根据Illumina测序说明，将10 pM文库变性为单链DNA分子，在Illumina flowcell上捕获，原位扩增为簇，并在Illumina HiSeq测序仪上采用双端模式（PE mode）进行150 cycle测序。

1.4　circRNA测序数据分析

通过Illumina HiSeq 4000测序仪测序，并使用circBase数据库和circ2Traits对所鉴定的环状RNA进行注释[12-13]。参考倍数变化（fold change，FC）和P值进行筛选，选择FC ≥ 2.0，P < 0.05作为筛选差异circRNA的阈值。circRNA高通量测序由上海云序生物有限公司完成检测及数据初步分析。

1.5　qRT-PCR、酶耐受及sanger测序验证

根据高通量测序结果，选取表达差异程度较显著的circRNA（FC越大，P值越小越佳；原始信号表达值越平均越佳），并结合功能分析结果，共选取10个circRNA，采用测序的6对样本进行qRT-PCR验证。对提取的RNA使用SuperScript Ⅲ逆转录试剂盒逆转录为cDNA文库。选取GAPDH为内参，使用ViiA 7实时荧光定量PCR仪，采用qPCR SYBR Green master mix试剂盒进行qRT-PCR。本研究所用引物序列由上海生工提供，引物序列见表2。采用$2^{-\Delta\Delta Ct}$法对目的基因的相对表达水平进行计算分析，得到各目的基因差异表达情况。根据PCR选取circRNA进行酶耐受实验，使用RNase处理提取的RNA，37 ℃水浴20 min，结果合格者进行sanger测序验证。另选OLK与NOM的样本组织各20例，对筛选合格circRNA circHLA-C进行qRT-PCR进行验证。

表2　qRT-PCR引物信息

Table 2　The primers used for qRT-PCR experiments

Circular RNA	Primer type	Primer sequence (5'-3')
CircHLA-C	Forward	CGGCAAGGATTACATCGC
(chr6:31238920-31324013-)	Reverse	CCTTCCCGTTCTCCAGGT
CircPLIN4	Forward	TGTGTGCAGTGGGGTGAC
(chr19:4511523-4511918-)	Reverse	CCCTTTGGCGACATTCAC
CircMTX2	Forward	TGACGCACTGGTATTTGGC
(chr2:177161588-177202305+)	Reverse	GCATTTTCAGGCCAAGGTT
CircRNF13	Forward	TGGGCATCTGTCTCATCTTG
(chr3:149613260-149639014+)	Reverse	GTCGTTGGATCCCATGCT
CircSENP2	Forward	CCTCAACAGCTGAATGGGA
(chr3:185293003-185344181+)	Reverse	CCCACATCTCCCCCTTCT
CircPLEKHM2	Forward	ACTCCGTCACCTCCACCA
(chr1:16044388-16047883+)	Reverse	CCGCAGGTAGCTCTCCAA
CircEMB	Forward	TGCTCAGCAGGAGCTTCA
(chr5:49694941-49707217-)	Reverse	CTGCATTCAAATCCCCAGA
CircERICH1	Forward	AAAACGCTGCTGCTCCTG
(chr8:618598-624047-)	Reverse	TCTTTGGCTGGTCATGAGG
CircALDH3A2	Forward	TCAAAGGTGGATTGGGGA
(chr17:19554860-19575269+)	Reverse	CATCCAGCATGGTGAGCA
CircZNF720	Forward	CGCTGTCTCTAAGCCGGA
(chr16:31733947-31734674+)	Reverse	GGTGTTCCCACTCCTCCC

1.6　ceRNA网络的构建及功能富集分析

通过TargetScan和miRanda预测目的circRNA下游的miRNA、mRNA，利用生物信息学软件Cytoscape（v2.8.0）构建ceRNA网络图。

对差异表达基因进行GO富集分析和KEGG功能通路分析，预测差异circRNA的功能和参与的功能通路。GO分析主要归类为三部分，包括：生物学过程、细胞成分和分子功能。GO分析是根据差异表达circRNA的来源基因和GO注释列表对GO条目进行筛选。

1.7　统计学方法

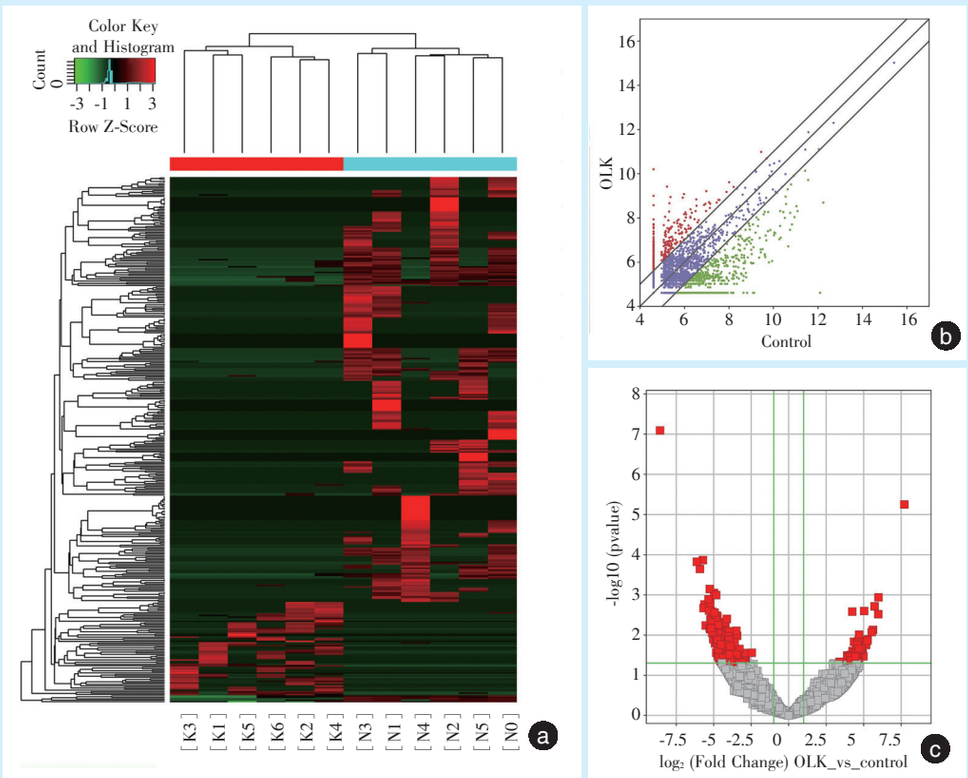
数据分析采用SPSS 19.0和Graphpad Prism 8.0完成。计量资料符合正态分布，用$\bar{x} \pm s$表示，两组间比较采用独立t检验。使用ROC曲线分析circHLA-C诊断的特异性与灵敏性，并用spearman等级

相关分析circHLA-C与OLK异常增生程度相关性。$P<0.05$为差异具有统计学意义。

2　结　果

2.1　OLK与NOM间差异circRNA表达谱及表达特点

聚类分析图显示了OLK与NOM间的差异circRNA表达谱（图1a）。根据统计标准，绘制散点图和火山图（图1b、1c）。在OLK中，共鉴定出366个显著差异的circRNA，其中65个上调，301个为下调。在这366个circRNA中，发现28个新的circRNA，其余的circRNA在circbase中已有过报道，且差异表达circRNA大多数为外显子circRNA（图2a、2b），提示差异circRNA具有重要的生物学功能。circRNA的长度集中在小于500 bp和500～2 000 bp两组内（图2c）。这些差异表达circRNA几乎分布在所有染色体上，包括22条常染色体和X染色体（图2d），提示差异circRNA分布较为广泛。



a: hierarchical clustering analysis showed circRNA expression profles that were different between OLK tissues and NOM tissues; red represents upregulated circRNAs, and green represents downregulated circRNAs; b: scatter plot of differences in circRNA expression between OLK and NOM tissues; red represents upregulated circRNAs with FC ≥ 2.0 in OLK tissues; green represents downregulated circRNAs with FC ≥ 2.0; c: volcano plot of signifcantly dysregulated circRNAs in OLK tissues; red represents eligible circRNAs (FC ≥ 2.0; $P<0.05$). OLK: oral leukoplakia; NOM: normal oral mucosal

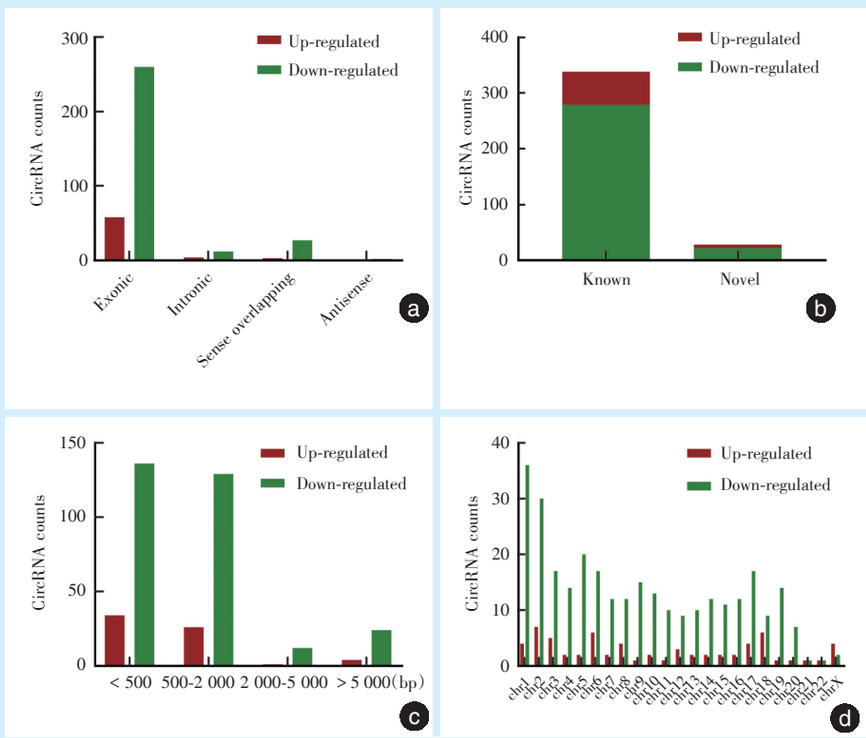
Figure 1　Expression profiles of circRNAs in OLK versus NOM tissues

图1　OLK与NOM组织中circRNA的表达谱

未经处理和分析的测序数据经标准化后，已上传至GEO网站，GEO序列号为GSE131568。

2.2　进一步验证目的circRNA

选取的10个circRNA包括4个表达上调和6个表达下调的circRNA（表3）。经验证，在这10个circRNA中有7个qRT-PCR结果与测序结果保持一致，且具有统计学意义（$P<0.05$，图3）。由于circRNA对核酸酶不敏感，通过qRT-PCR验证的7个circRNA中选择了5个疾病相关性高的circRNA进行酶耐受实验。结果表示，circHLA-C、circPLIN4（perilip3-4）和circRNF13可抵抗核糖核酸酶的水解（图3c），对这3个circRNA进行Sanger测序。结



a: significantly differentially expressed circRNAs were divided into 4 types according to the host gene structure(exon, intron, antisense, and sense overlapping circRNA); b: filtered circRNAs were classified by whether they were newly discovered; c: classification according to the circRNA length; d: chromosome distribution of upregulated and downregulated signifcantly differentially expressed circRNAs

Figure 2　Distribution of the characteristics of significantly dysregulated circRNAs

图2　显著表达差异circRNA分布特点

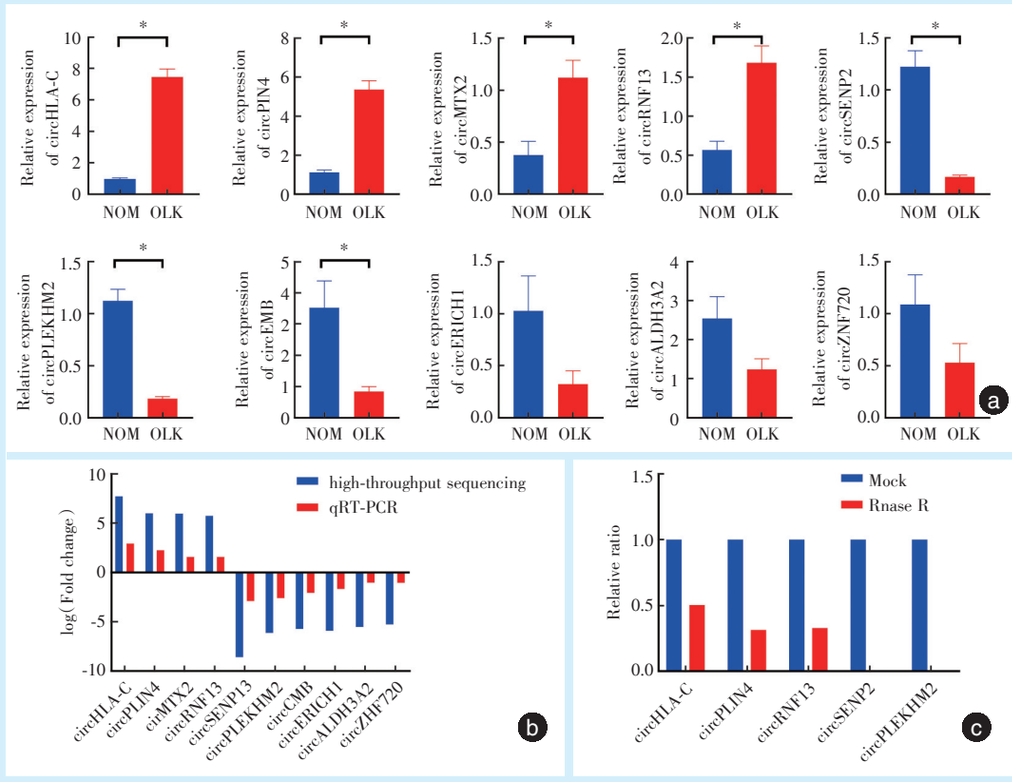
果显示,差异表达最显著的circHLA-C是真正的circRNA,且具有特定反式剪接位点。进一步对circHLA-C进行了可视化分析,circHLA-C由8个外显子和7个内含子组成,且反剪接位点为CTACCTGG(图4)。

表3　OLK组织10个显著表达差异circRNA

Table 3　Ten circRNAs differentially expressed in OLK

CircRNA ID	FC	*P*	Chromosome	Strand	Gene name	Sequence length(bp)
CircHLA-C(chr6:31238920-31324013-)	7.719	< 0.001	chr6	-	HLA-C	85 094
CircPLIN4(chr19:4511523-4511918-)	5.992	0.001	chr19	-	PLIN4	396
CircMTX2(chr2:177161588-177202305+)	5.980	0.003	chr2	+	MTX2	40 718
CircRNF13(chr3:149613260-149639014+)	5.735	0.002	chr3	+	RNF13	379
CircSENP2(chr3:185293003-185344181+)	-8.583	< 0.001	chr3	+	SENP2	51 179
CircPLEKHM2(chr1:16044388-16047883+)	-6.122	< 0.001	chr1	+	PLEKHM2	435
CircERICH1(chr8:618598-624047-)	-5.917	< 0.001	chr8	-	ERICH1	954
CircEMB(chr5:49694941-49707217-)	-5.722	< 0.001	chr5	-	EMB	12 277
CircALDH3A2(chr17:19554860-19575269+)	-5.532	0.006	chr17	+	ALDH3A2	1 290
CircZNF720(chr16:31733947-31734674+)	-5.258	0.001	chr16	+	ZNF720	223

OLK: oral leukoplakia; FC: fold change; HLA-C: human leukocyte antigen-C; PLIN4: perilipin-4; MTX2: metaxin 2; RNF13: ring finger protein 13; SENP2: sentrin/SUMO-specific proteases; PLEKHM2: pleckstrin homology and RUN domain containing M2; ERICH1: glutamate rich 1; EMB: embigin; ALDH3A2: aldehyde dehydrogenase 3 family member A2; ZNF720: zinc finger protein 720



a: ten selected circRNAs were subjected to qRT-PCR validation in 6 NOM and 6 OLK tissues; b: comparison of the results of high-throughput sequencing and qRT-PCR. c: RNase R analysis showed that circHLA-C, circPLIN4 and circRNF13 resisted digestion by RNase R; *: $P < 0.05$. OLK: oral leukoplakia; FC: fold change; HLA-C: human leukocyte antigen-C; PLIN4: perilipin-4; MTX2: metaxin 2; RNF13: ring finger protein 13; SENP2: sentrin/SUMO-specific proteases; PLEKHM2: pleckstrin homology and RUN domain containing M2; ERICH1: glutamate rich 1; EMB: embigin; ALDH3A2: aldehyde dehydrogenase 3 family member A2; ZNF720: zinc finger protein 720

Figure 3　Ten circRNAs differentially expressed in OLK

图3　OLK组织10个显著差异表达circRNA

2.3　OLK中circRNA的功能富集

在OLK表达上调的circRNA中，circHLA-C的来源基因HLA-C富集到多达95个GO功能条目，分别包括44条生物学过程、45条细胞组分和6条分子功能通路（见本文OSID码）。HLA-C在生物学过程中可参与调控T细胞介导的细胞毒性和免疫功能。在细胞组分功能富集分析中，发现HLA-C可调控Ⅰ类人类主要组织相容复合体（major histocompatibility complex，MHC）。

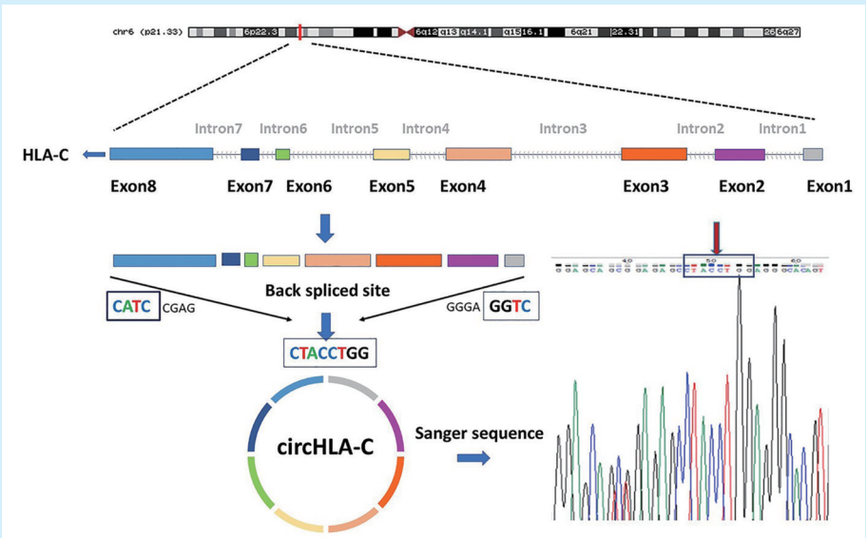
通过来源基因的相关通路，进行KEGG通路富集分析，预测circRNA功能通路。与366个差异基因相关的KEGG通路共有139条，其中富集到上调的circRNA通路有60条。OLK作为一种癌前病变，在上调通路中共预测到3条KEGG通路参与癌变过程。肿瘤相关蛋白聚糖、肿瘤相关miRNA和肿瘤相关通路都可参与调节胞外信号蛋白激酶/促分裂原活化蛋白激酶（extracellular-signal-regulated protein kinase/mitogen-activated protein kinase，ERK/MAPK1），二者已被证明是OLK发病的关键因素[14-15]。301个下调circRNA参与的79条KEGG通路，其中显著调节的有泛素介导的蛋白水解、调节干细胞多能性的信号通路和mTOR信号通路（图5）。

2.4　ROC曲线分析circHLA-C对OLK的诊断意义

通过ROC曲线分析评估circHLA-C对OLK的诊断意义。circHLA-C曲线下面积（Area Under Curve，AUC）为0.955（95% *CI*：0.892～1.00，$P < 0.001$）（图6）。提示circHLA-C具有成为OLK生物标志物的可能性。

2.5　circHLA-C与OLK异常增生的相关性

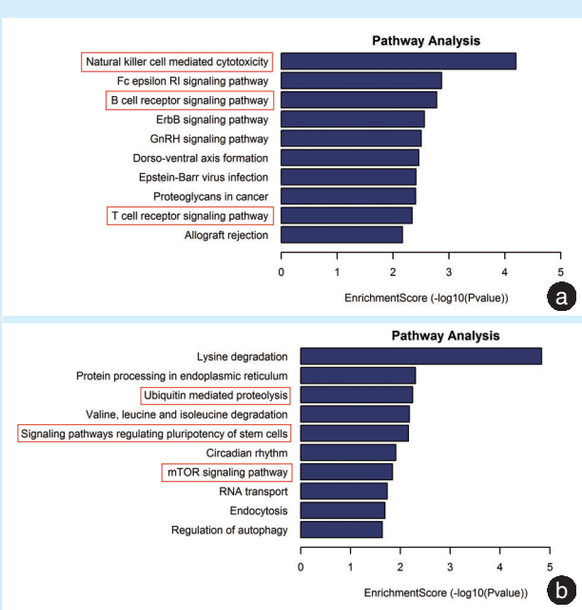
分析20例OLK组织circHLA-C的表达量与异常



HLA-C: human leukocyte antigen-C

Figure 4　Ring formation mechanism and back spliced site of circHLA-C

图4　circHLA-C的成环机制及反剪接位点



a: top 10 in the KEGG analysis of upregulated circRNAs; b: top 10 in the KEGG analysis of downregulated circRNAs. The terms in the red frame indicate important pathways in OLK; OLK: oral leukoplakia

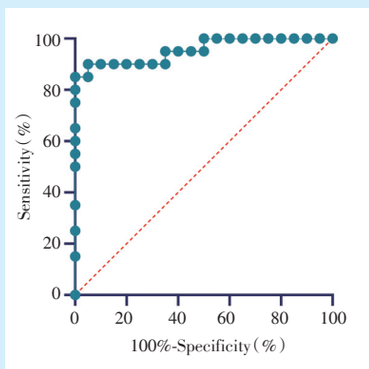
Figure 5　KEGG analysis of the differential expression of circRNA in OLK

图5　KEGG分析OLK中差异表达circRNA

增生程度的相关性，结果表明circHLA-C表达量与OLK轻、中度异常增生呈正相关($r=0.478$, $P=0.033$)。

2.6　ceRNA网络分析

本研究共筛选出7个circRNA建立ceRNA网络（图7）。根据结合能力，列出了每个circRNA调控的前5个miRNA。生物信息学继续分析下游的miRNA-mRNA网络，图中呈现了前5个miRNA能在下游抑制的mRNA。这表明circRNA具有十分广泛的作用空间，可通过多种不同的通路对下游分子甚至是蛋白进行调控。



OLK: oral leukoplakia

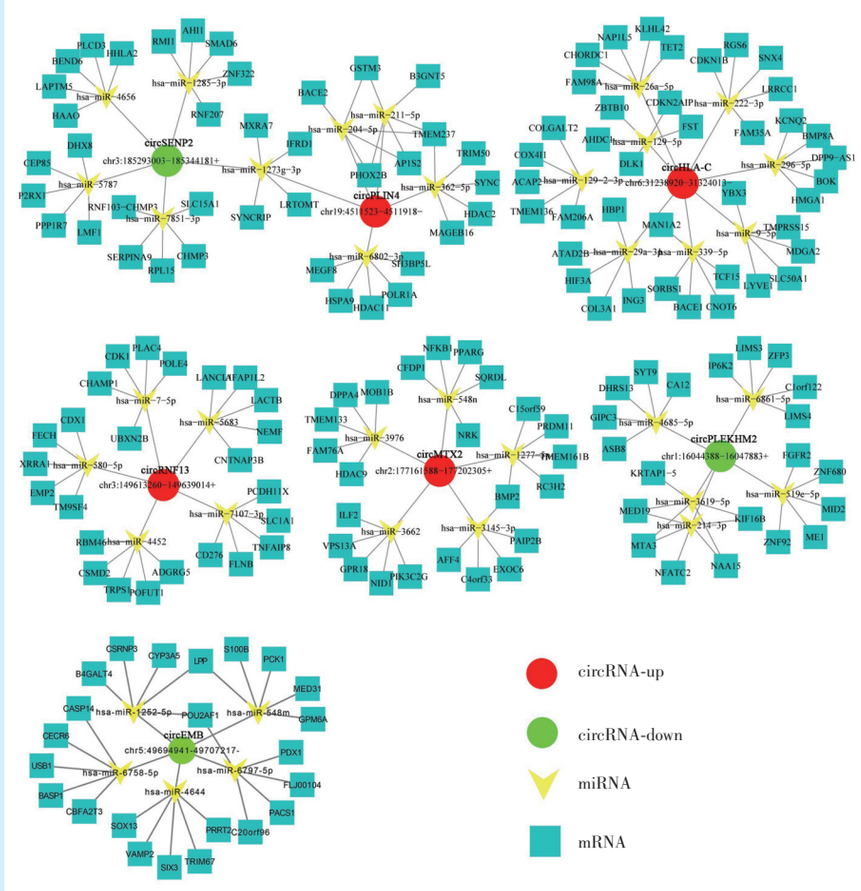
Figure 6　ROC curve analysis of circHLA-C in OLK

图6　circHLA-C在OLK中的ROC曲线分析

3　讨　论

OLK是最常见的口腔黏膜潜在恶性病变，研究表明OLK发生癌变平均需要4至5年[16-17]，因此，OLK的早期诊断和治疗非常重要。circRNA曾被认为是分子内错剪接的副产物[18]。近年来，circRNA得到越来越多科研界研究者的关注，目前circRNA已在真核细胞内确认是一类新的广泛存在、丰度高、保守性高且较为稳定的内源性非编码RNA[19-21]。研究表明circRNA_100290，circDOCK1和circ_0007059可通过ceRNA机制调控口腔鳞状细胞癌（oral squamous carcinoma cell，OSCC）的生长和转移[22-24]。hsa_circ_0005320，hsa_circ_0067531和hsa_circ_0000869可调控口腔黑色素瘤的形成和转移[25]。

circRNA可作为顺式作用元件调控其亲本基因的表达，促进基因转录[26]。本研究通过circRNA来源基因的功能预测circRNA的功能。HLA-C表达的变化可影响免疫系统对感染性和自身免疫性疾病的应答效果，且HLA-C的进化使其与杀伤细胞免疫球蛋白样受体的相互作用更加强烈，从而影响自然杀伤细胞反应的调节[27]。研究表明KIR2DL1(+)-HLA-C2(+)基因型可能是OSCC早期易感人群的危险因素[28]。



ceRNA：competing endogenous RNA

Figure 7　CeRNA interaction

图7　CeRNA网络图

GO功能富集分析显示差异表达circRNA具有丰富的功能，多数差异表达circRNA都可富集到细胞周期过程。推测调控细胞周期的基因表达的变化是OLK发生的主要原因之一。HLA-C可富集到功能T细胞介导的细胞毒性和免疫、MHCⅠ类蛋白复合体。研究表明，MHCⅠ类分子与特异性T细胞受体和$CD8^+$细胞表面分子的识别有关。MHCⅠ类分子是OLK中的关键分子，在OLK中呈低表达，且与异常增生程度相关[29-30]。此外，OSCC和伴异常增生OLK中$CD8^+$细胞数量明显多于无异常增生的OLK[31]。

KEGG通路富集分析显示在60种上调的circRNA通路中，富集分数排名前10通路包括自然杀伤细胞介导的细胞毒性、B细胞受体信号通路、EB病毒感染、蛋白多糖在癌症中的作用、T细胞受体信号通路和移植物排斥等。在这些途径中，circHLA-C的靶基因参与了自然杀伤细胞介导的细胞毒性、EB病毒感染和移植物排斥。这一结果提示OLK可能与感染和免疫抑制因子有关，而circHLA-C可能是其中的关键因素。与NOM组织样本相比OLK中circHLA-C显著升高。ROC曲线表明circHLA-C对OLK的诊断具有较高的特异性和灵敏性，且circHLA-C的表达量与轻、中度异常增生程度呈正相关。揭示了circHLA-C可能成为新的生物标志物。

研究表明circRNA可作为ceRNA作用于细胞调控网络[32]。本研究构建的ceRNA网络显示circHLA-C可通过海绵机制与miR-9、miR-339-5p、miR-29a-3p、miR-26a-5p、miR-222-3p进行互补配对，通过TargetScan、MiRanda软件可预测circHLA-C与以上miRNA之间具有结合位点。有研究发现OLK和OSCC患者血清miR-9表达较NOM标本明显降低[33]。在OLK和一些原发癌组织中，miR-339-5p表达下调，作为肿瘤抑制因子参与癌症进展[34-35]。miR-29a-3p和miR-26a-5p在OLK和癌组织中显著下调[36]。此外，与正常和OSCC患者相比，OLK中miR-222-3p显著下调[37]。由此可见，circHLA-C可能具有同一miRNA的多个结合位点或多个不同miRNA的结合位点。circHLA-C可能就是通过海绵吸附这些miRNAs来调控OLK的发生发展。对于circHLA-C与miRNAs及OLK下游靶基因的相互作用，今后还需要进行更深入的研究。

综上所述，本研究探讨了circRNA在OLK病因病理及发生发展中的作用，并揭示了circHLA-C具有成为OLK潜在分子标志物及治疗靶点的可能。

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参考文献

[1] Hong WK, Endicott J, Itri LM, et al. 13-cis-retinoic acid in the treatment of oral leukoplakia[J]. N Engl J Med, 1986, 315(24): 1501-1505. doi: 10.1056/NEJM198612113152401.

[2] Memczak S, Jens M, Elefsinioti A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency[J]. Nature, 2013, 495(7441): 333-338. doi: 10.1038/nature11928.

[3] Bach DH, Lee SK, Sood AK. Circular RNAs in cancer[J]. Mol Ther Nucleic Acids, 2019, 16: 118-129. doi: 10.1016/j.omtn.2019.02.005.

[4] Altesha MA, Ni T, Khan A, et al. Circular RNA in cardiovascular disease[J]. J Cell Physiol, 2019, 234(5): 5588-5600. doi: 10.1002/jcp.27384.

[5] Zhong Y, Du Y, Yang X, et al. Circular RNAs function as ceRNAs to regulate and control human cancer progression[J]. Mol Cancer, 2018, 17(1): 79. doi: 10.1186/s12943-018-0827-8.

[6] Kristensen LS, Andersen MS, Stagsted LV, et al. The biogenesis, biology and characterization of circular RNAs[J]. Nat Rev Genet, 2019, 20(11): 675-691. doi: 10.1038/s41576-019-0158-7.

[7] Zhang X, Wang S, Wang H, et al. Circular RNA circNRIP1 acts as a microRNA-149-5p sponge to promote gastric cancer progression *via* the AKT1/mTOR pathway[J]. Mol Cancer, 2019, 18(1): 20. doi: 10.1186/s12943-018-0935-5.

[8] Li X, Yang L, Biogenesis CT, et al. And challenges of circular RNAs[J]. Mol Cell, 2018, 71(3): 428-442. doi:10.1016/j.molcel.2018.06.034.

[9] Wilusz JE. A 360° view of circular RNAs: from biogenesis to functions[J]. Wiley Interdiscip Rev RNA, 2018, 9(4): e1478. doi: 10.1002/wrna.1478.

[10] Chen B, Huang S. Circular RNA: an emerging non-coding RNA as a regulator and biomarker in cancer[J]. Cancer Lett, 2018, 418: 41-50. doi: 10.1016/j.canlet.2018.01.011.

[11] Xu S, Song Y, Shao Y, et al. Comprehensive analysis of circular RNA in oral leukoplakia: upregulated circHLA-C as a potential biomarker for diagnosis and prognosis[J]. Ann Transl Med, 2020, 8(21): 1375. doi: 10.21037/atm-20-3840.

[12] Glažar P, Papavasileiou P, Rajewsky N. CircBase: a database for circular RNAs[J]. RNA, 2014, 20(11): 1666-1670. doi: 10.1261/rna.043687.113.

[13] Ghosal S, Das S, Sen R, et al. Circ2traits: a comprehensive database for circular RNA potentially associated with disease and traits [J]. Front Genet, 2013, 4: 283. doi: 10.3389/fgene.2013.00283.

[14] Li Z, Gao M, Liu C, et al. Immunohistochemical detection of aurora a and ERK pathway in oral leukoplakia and oral squamous cell carcinoma[J]. J Hard Tissue Biol, 2014, 23(1): 71-76. doi:10.2485/

jhtb.23.71.

[15] Yang W, Wang XM, Yuan HY, et al. Exploring the mechanism of WWOX growth inhibitory effects on oral squamous cell carcinoma [J]. Oncol Lett, 2017, 13(5): 3198-3204. doi: 10.3892/ol.2017.5850.

[16] Neville BW, Day TA. Oral cancer and precancerous lesions[J]. CA Cancer J Clin, 2002, 52(4): 195-215. doi: 10.3322/canjclin.52.4.195.

[17] Brouns E, Baart J, Karagozoglu K, et al. Malignant transformation of oral leukoplakia in a well-defined cohort of 144 patients[J]. Oral Dis, 2014, 20(3): e19-e24. doi: 10.1111/odi.12095.

[18] Cocquerelle C, Mascrez B, Hétuin D, et al. Mis-splicing yields circular RNA molecules[J]. FASEB J, 1993, 7(1): 155-160.

[19] Zheng Q, Bao C, Guo W, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs[J]. Nat Commun, 2016, 7(1): 11215. doi: 10.1038/ncomms11215.

[20] Wang YF, Li BW, Sun S, et al. Circular RNA expression in oral squamous cell carcinoma[J]. Front Oncol, 2018, 8: 398. doi: 10.3389/fonc.2018.00398.

[21] Qiu X, Ke X, Ma H, et al. Profiling and bioinformatics analyses reveal differential expression of circular RNA in tongue cancer revealed by high-throughput sequencing[J]. J Cell Biochem, 2019, 120(3): 4102-4112. doi: 10.1002/jcb.27695.

[22] Chen X, Yu J, Tian H, et al. Circle RNA hsa_circRNA_100290 serves as a ceRNA for miR-378a to regulate oral squamous cell carcinoma cells growth via Glucose transporter-1 (GLUT1) and glycolysis[J]. J Cell Physiol, 2019, 234(11): 19130 - 19140. doi: 10.1002/jcp.28692.

[23] Wang L, Wei Y, Yan Y, et al. CircDOCK1 suppresses cell apoptosis via inhibition of miR-196a-5p by targeting BIRC3 in OSCC[J]. Oncol Rep, 2018, 39(3): 951-966. doi: 10.3892/or.2017.6174.

[24] Su W, Wang Y, Wang F, et al. Circular RNA hsa_circ_0007059 indicates prognosis and influences malignant behavior via AKT/mTOR in oral squamous cell carcinoma[J]. J Cell Physiol, 2019, 234(9): 15156-15166. doi: 10.1002/jcp.28156.

[25] Ju H, Zhang L, Mao L, et al. Altered expression pattern of circular RNAs in metastatic oral mucosal melanoma[J]. Am J Cancer Res, 2018, 8(9): 1788-1800.

[26] Li Z, Huang C, Bao C, et al. Exon-intron circular RNAs regulate transcription in the nucleus[J]. Nat Struct Mol Biol, 2015, 22(3): 256-264. doi: 10.1038/nsmb.2959.

[27] Kaur G, Gras S, Mobbs J, et al. Structural and regulatory diversity shape HLA-C protein expression levels[J]. Nat Commun, 2017, 8: 15924. doi: 10.1038/ncomms15924.

[28] Dutta A, Saikia N, Phookan J, et al. Association of killer cell immunoglobulin-like receptor gene 2DL1 and its HLA-C2 ligand with family history of cancer in oral squamous cell carcinoma[J]. Immunogenetics, 2014, 66(7/8): 439-448. doi: 10.1007/s00251-014-0778-1.

[29] 吴平凡, 韩帮锋, 夏辉, 等. 人类主要组织相容抗原Ⅰ类分子在口腔白斑中表达的临床意义[J]. 华西口腔医学杂志, 2010, 28(4): 439-442.
Wu PF, Han BF, Xia H, et al. The expression of human major histocompatibility complex-I in oral leukoplakia[J]. Hua Xi Kou Qiang Yi Xue Za Zhi, 2010, 28(4): 439-442.

[30] Prime SS, Pitigala-Arachchi A, Crane IJ, et al. The expression of cell surface MHC class I heavy and light chain molecules in premalignant and malignant lesions of the oral mucosa[J]. Histopathology, 1987, 11(1): 81-91. doi: 10.1111/j.1365-2559.1987.tb02611.x.

[31] Ohman J, Magnusson B, Telemo E, et al. Langerhans cells and T cells sense cell dysplasia in oral leukoplakias and oral squamous cell carcinomas--evidence for immunosurveillance[J]. Scand J Immunol, 2012, 76(1): 39-48. doi:10.1111/j.1365-3083.2012.02701.x.

[32] Liang YW, Zhang YZ, Xu LA, et al. CircRNA expression pattern and ceRNA and miRNA-mRNA networks involved in anther development in the CMS line of brassica campestris[J]. Int J Mol Sci, 2019, 20(19): 4808. doi: 10.3390/ijms20194808.

[33] Sun L, Liu L, Fu H, et al. Association of decreased expression of serum miR-9 with poor prognosis of oral squamous cell carcinoma patients[J]. Med Sci Monit, 2016, 22: 289-294. doi: 10.12659/msm.895683.

[34] Maimaiti A, Abudoukeremu K, Tie L, et al. MicroRNA expression profiling and functional annotation analysis of their targets associated with the malignant transformation of oral leukoplakia[J]. Gene, 2015, 558(2): 271-277. doi: 10.1016/j.gene.2015.01.004.

[35] Zhang L, Lu XQ, Zhou XQ, et al. NEAT1 induces osteosarcoma development by modulating the miR-339-5p/TGF-β1 pathway[J]. J Cell Physiol, 2019, 234(4): 5097-5105. doi: 10.1002/jcp.27313.

[36] Roy R, Singh R, Chattopadhyay E, et al. MicroRNA and target gene expression based clustering of oral cancer, precancer and normal tissues[J]. Gene, 2016, 593(1): 58-63. doi: 10.1016/j.gene.2016.08.011.

[37] Chang Y, Weng SL, Yang SF, et al. A three-microRNA signature as a potential biomarker for the early detection of oral cancer[J]. Int J Mol Sci, 2018, 19(3): 758. doi: 10.3390/ijms19030758.

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