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• 基础研究 •

原代人口腔角质细胞在产黑普氏菌作用后的转录组分析

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【摘要】 目的 探究产黑普氏菌(*Prevotella melaninogenica*, *P.m*)作用下原代人口腔角质细胞(primary human oral keratinocytes, pHOK)的转录组变化,并在口腔角质形成细胞(human oral keratinocyte, HOK)细胞系中进行验证。**方法** 分离培养pHOK,并与*P.m*共培养4 h与24 h,提取总RNA,构建基因文库,转录组测序,分析差异表达基因,并进行基因本体论(gene ontology, GO)通路分析与京都基因与基因组百科全书(Kyoto Encyclopedia of Genes and Genomes, KEGG)通路分析,在HOK与*P.m*共培养模型中采用qRT-PCR及Western Blot对差异基因进行验证。**结果** 在pHOK与*P.m*共培养4 h组与对照组间上调表达的差异基因有:淋巴细胞胞浆蛋白1(lymphocyte cytosolic protein 1, LCP1)、角蛋白7(keratin 7, KRT7)、纤毛和鞭毛相关蛋白251(cilia and flagella associated protein 251, CFAP251)等,下调表达的差异基因有:含FERM、RhoGEF和Pleckstrin结构域蛋白1(FERM, ARH/RhoGEF and Pleckstrin domain protein 1, FARP1)、WW结构域转录调控因子1(WW domain containing transcription regulator 1, WWTR1)、含Discoidin、CUB和LCCL结构域蛋白2(Discoidin, CUB and LCCL domain-containing protein 2, DCBLD2)等,共1 788个差异表达基因;24 h组与对照组间上调表达的差异基因有:LCP1、补体C1s(complement C1s, C1S)、犬尿氨酸酶(kynureninase, KYNU)等,下调表达的差异基因有:磷酸丝氨酸转氨酶-1(phosphoserine aminotransferase 1, PSAT1)、FARP1、FKBP型脯氨酰异构酶10(FKBP prolyl isomerase 10, FKBP10)等,共1 832个差异表达基因;其中共同差异表达基因(common differentially expressed genes, cDEGs)有LCP1、KYNU、长链非编码RNA958(long intergenic non-protein coding RNA 958, LINC00958)等1 090个,差异均具有统计学意义。通过GO数据库分析,cDEGs主要富集到细菌对脂多糖的反应、对细菌来源分子的反应、细胞的顶端部分等;通过KEGG分析,cDEGs富集到的通路有白介素-17信号通路、肿瘤坏死因子信号通路、Toll样受体通路等;从cDEGs中筛选肌球蛋白1B(myosin1B, MYO1B)在HOK与*P.m*共培养模型中进行验证,qRT-PCR及Western Blot检测结果表明,MYO1B的表达在对照组和*P.m*刺激组之间存在显著性差异($P < 0.001$),且其表达随*P.m*刺激时间的延长及刺激浓度的增大而递增。**结论** *P.m*对口腔角质细胞的转录组存在重要影响。

【关键词】 口腔扁平苔藓; 产黑普氏菌; 转录组测序; 肌球蛋白1B; 上皮屏障功能; 白介素-17信号通路; 肿瘤坏死因子信号通路; Toll样受体通路; 细胞顶端部分

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Transcriptome analysis of primary human oral keratinocytes stimulated with *Prevotella melaninogenica*

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【Abstract】 Objective To investigate the transcriptomic changes in primary human oral keratinocytes (pHOKs) after coculture with *Prevotella melaninogenica* (*P.m*) and to verify the changes in human oral keratinocyte (HOK) cell lines. **Methods** pHOK was isolated and cocultured with *P.m* for 0, 4 and 24 h. Total RNA was extracted, a gene library was constructed, transcriptional sequencing was performed, differentially expressed genes (DEGs) were analyzed, gene ontology (GO) pathway analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed, and the validation of DEGs was performed by qRT-PCR and Western Blot in the HOK and *P.m* coculture cell model. **Results** 1 788 DEGs were detected between the 4 h group and control group, including upregulated DEGs such as lymphocyte cytosolic protein 1(LCP1), keratin 7 (KRT7) and Cilia and flagella associated protein 251(CFAP251) and downregulated DEGs such as FERM, ARH/RhoGEF and Pleckstrin domain protein 1 (FARP1), WW domain containing transcription regulator 1(WWTR1) and Discoidin, CUB and LCCL domain-containing protein 2 (DCBLD2). 1 832 DEGs were detected between the 24 h group and control group, including upregulated DEGs such as LCP1, complement C1s(C1S), kynureninase (KYNU) and downregulated DEGs such as phosphoserine aminotransferase 1 (PSAT1), FARP1 and FKBP prolyl isomerase 10 (FKBP10). There were 1 090 common differentially expressed genes (cDEGs) in the 4 h and 24 h groups, including LCP1, KYNU and long intergenic nonprotein coding RNA 958 (LINC00958). The GO pathways were mainly enriched in response to lipopolysaccharide and the molecules of bacterial origin and apical part of the cell. KEGG pathway analysis revealed enrichment in the interleukin-17 (IL-17) signaling pathway, tumor necrosis factor (TNF) signaling pathway, Toll-like receptor (TLR) pathway, etc. We verified the expression of a cDEG, Myosin1B (MYO1B), and qRT-PCR and Western Blot analysis showed that MYO1B expression was significantly upregulated between the control group and the *P.m* cocultured group ($P < 0.001$), and its expression followed a time-dependent and concentration-dependent manner. **Conclusion** *P.m* played an important role in the transcriptome of oral keratinocytes.

【Key words】 oral lichen planus; *Prevotella melaninogenica*; RNA-seq; myosin1B; epithelial barrier function; interleukin-17 signaling pathway; tumor necrosis factor signaling pathway; Toll-like receptor pathway; apical part of cell

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口腔扁平苔藓(oral lichen planus, OLP)是口腔黏膜病专科中最常见的疾病之一,最新研究表明整体发病率在0.89%左右^[1]。目前OLP发病机制尚不明确,普遍认为OLP是机体内在与外在因素联合作用引发的疾病。在一些外在因素(病原体、药物、牙科材料等)作用下,易感人群口腔黏膜病损部位的上皮细胞与抗原呈递细胞活化,这些细胞分泌多种炎症因子与趋化因子,激活T淋巴细胞,形成持续存在的免疫炎症反应^[2]。在OLP患者的唾液、组织及颊黏膜拭子中发现了菌群失调的现象^[3-5],菌群失调可能在OLP发生发展过程中起到重要作用^[6]。本课题组前期研究发现,OLP组颊黏膜表面微生物菌落结构与健康对照组相比发生显著性改变,其中产黑普氏菌(*Prevotella melaninogenica*, *P.m*)在OLP组颊黏膜表面构成比显著增加,且该菌能够侵入到OLP病损组织上皮层及固有层,并与巨噬细胞相互作用,提示*P.m*可能在

OLP的发生发展中起到重要作用^[5]。而口腔黏膜上皮角质形成细胞作为抵抗外界入侵的第一道屏障,*P.m*与口腔上皮角质细胞的相互作用并不清楚。

转录组测序是对特定细胞在某一功能状态下所能转录出来的所有RNA的总和进行高通量测序。通过新一代高通量测序,能够全面快速地获得某一物种特定组织或器官在某一状态下的几乎所有转录本序列信息。目前,对OLP转录组研究并不完善,多集中于OLP的癌变倾向^[7]、OLP的炎症反应过程^[8]等。有关口腔共生菌作用下,口腔上皮角质形成细胞转录组的改变目前尚无研究。因此本研究利用转录组测序技术,研究原代人口腔角质形成细胞(primary human oral keratinocytes, pHOKs)在*P.m*刺激下基因表达的改变,对差异表达基因(differentially expressed genes, DEGs)进行生物信息学分析,并筛选其中的差异基因进行验证,

为探究 DEGs 所参与的通路 with OLP 的相关性提供实验基础。

1 材料和方法

1.1 主要材料和仪器

人口腔角质形成细胞系 (human oral keratinocyte, HOK) 由陈谦明教授惠赠^[9]。Prevotella melaninogenica ATCC® 25845™ (ATCC, 美国), OKM 培养基 (Sciencell, 美国), PBS (上海生工, 中国), dispase 酶 (Sigma, 美国), DMEM 培养基 (Hyclone, 美国), 胎牛血清 (WISSENT, 美国), RNAiso Plus (TaKaRa, 中国), 无水乙醇 (上海生工, 中国), 氯仿 (国药, 中国), 异丙醇 (国药, 中国), PrimeScript™ RT reagent-Kit Perfect Real Time (TaKaRa, 中国), TB Green Premix Ex Taq (TaKaRa, 中国), BCA protein assay kit (Solarbio, 中国), MYO1B 一抗 (Santa Cruz, 美国), β -Tubulin 一抗 (Proteintech Biotechnology, 中国), 辣根过氧化物酶标记羊抗兔 IgG (碧云天, 中国), 超敏 ECL 化学发光试剂盒 (碧云天, 中国), 实时荧光定量 PCR 仪 (Thermo Fisher, 美国), 小型高速离心机 (Eppendorf, 美国), Bio-Rad 电泳系统 (Bio-Rad, 美国), 多功能酶标仪 (Bio-Tek, 美国)。

1.2 样本的收集与处理

1.2.1 pHOKs 的分离与培养 本实验获得同济大学附属口腔医院伦理委员会的批准 (伦理审批号: 2018-002)。组织来源于同济大学附属口腔医院口腔颌面外科智齿拔除术时取下的颊黏膜组织, 把组织放到含 10% 双抗 PBS 中清洗三遍。随后将组织放到 0.25% 中性蛋白酶 (dispase II) 中 4 ℃ 过夜。第二天用无菌眼科镊分离组织上皮, PBS 清洗 2 次, 0.25% 含 EDTA 的胰酶 37 ℃ 震荡消化 4 min, 用含 10% FBS 的 DMEM 培养基终止消化, 离心, 去上清, PBS 清洗沉淀 2 次, 用 2 mL OKM 培养基重悬沉淀并铺板于六孔板, 培养于 37 ℃、5% 体积分数 CO₂ 的细胞培养箱中。

1.2.2 HOK 细胞培养 HOK 细胞以 5×10^5 个/mL 的细胞密度接种于 12 孔板中, 在添加了 10% FBS 的 DMEM 培养基中, 37 ℃、5% 体积分数 CO₂ 的细胞培养箱中培养, 待镜下细胞密度达 90% 时用于实验。

1.2.3 P.m 菌株培养与菌液制备 冻存于 -80 ℃ 的 P.m 菌液于 37 ℃ 化冻, 均匀涂于基础厌氧血平板, 加厌氧袋置于密封袋, 置于 37 ℃ 孵箱复苏; 3 d 后挑取平板上黑褐色菌点于新鲜液体厌氧培养基中, 盖上并旋松管盖, 加厌氧袋置于密封袋中, 置

于 37 ℃ 摇床。1 d 后测菌液 OD₆₀₀=1, 以 4 000 rpm 离心 3 min, 弃上清, PBS 清洗 3 次后, 加 1 mL PBS 重悬菌液, 最终菌液浓度为 1×10^9 CFU/mL。

1.2.4 菌液与细胞共同培养 ①菌液与 pHOKs 共同培养: pHOKs 培养到第三代后用于实验, 吸去细胞上清液, PBS 轻柔洗涤细胞 3 次, 更换为不含血清的 DMEM 培养基, 每孔加入 10 μ L 菌液, 分别共培养 4 h 和 24 h 作为两实验组, 以未与菌液共培养的 pHOKs 作为对照组, 处理后的细胞于 37 ℃、5% 体积分数 CO₂ 的细胞培养箱中分别培养相应时长, 随后提取细胞总 RNA 用于转录组测序。

②菌液与 HOK 细胞系共同培养: HOK 细胞吸去细胞上清液, PBS 轻柔洗涤细胞 3 次, 更换为不含 FBS 的 DMEM 培养基。

为分别探究 P.m 对 HOK 细胞系在时间梯度和浓度梯度共培养后造成的影响, 设置时间梯度共培养组和浓度梯度共培养组, 以未与菌液共培养的 HOK 细胞作为对照组。时间梯度组每孔加入 10 μ L 菌液, 各组共培养体系最终菌液浓度为 5×10^6 CFU/mL, 分别培养 4 h、6 h、8 h; 浓度梯度组每孔分别加入 10 μ L、20 μ L、30 μ L 菌液, 各实验组体系中最终菌液浓度分别为: 5×10^6 CFU/mL、 10×10^6 CFU/mL、 15×10^6 CFU/mL, 共培养 4 h。用 DMEM 培养基补足液体量, 对照组只添加基础 DMEM 培养基, 使各组共培养体系最终液体的量相同, 总体积 2 mL, 以便计算菌液浓度。将处理后的细胞在 37 ℃、5% CO₂ 的细胞培养箱中培养, 随后吸去上清, PBS 轻柔洗细胞 3 次, 待后续实验。

1.3 CCK-8 法检测细胞活性

通过 CCK-8 法检测 P.m 刺激后 HOK 细胞的活性。HOK 细胞与 P.m 共培养。共培养完成后, 轻柔吸去细胞上清液, PBS 轻柔洗涤细胞 3 次, 每孔加入 100 μ L DMEM 培养基和 10 μ L CCK-8 溶液, 避光反应 1 h。随后在酶标仪 450 nm 处测定细胞上清液吸光度, 相对细胞活力计算方法: (实验组细胞吸光度/对照组细胞吸光度) $\times$ 100%。

1.4 总 RNA 的提取

将共培养完成后的细胞吸去上清, PBS 轻柔洗涤 3 次, 按试剂盒说明提取总 RNA。测定 RNA 浓度。

1.5 转录组测序

将样本送至上海晶能生物科技有限公司完成转录组测序。基本程序为: RNA 片段化, 逆转录成双链 DNA, 加工双链 DNA 末端, PCR 扩增。PCR 产物热变性形成单链, 单链 DNA 环化得到单链环状

DNA文库, DNA文库用Illumina Nova 6000测序平台进行高通量转录组测序, 获得高质量的转录组数据。

1.6 测序结果的分析

对数据质量评估, 对所获得的序列进行基因注释, 用HTSeq软件计算各个基因的reads count。使用R语言limma包进行DEGs挑选。取 $\log_2$ (差异倍数) ≥ 1 且 $P \leq 0.05$ 的基因筛选为具有统计学意义的DEGs。通过R软件的Pheatmap包对DEGs进行聚类分析, 用GO数据库对DEGs进行生物学功能注释, 用KEGG Pathway数据库对DEGs进行通路的注释。利用R语言富集常用包clusterProfiler(版本3.18.0)对DEGs做GO、KEGG pathway的注释富集分析。对DEGs数量 ≥ 2 , 且统计 $P \leq 0.05$ 的注释条目认为具有统计学意义。

1.7 qRT-PCR验证差异表达基因

逆转录试剂盒逆转录合成cDNA, 再以实时荧光定量试剂盒进行实时荧光定量PCR。以GAPDH为内参。肌球蛋白1B(myosin1B, MYO1B)正义链: 5'-GGAGACCATGGCCA AAATGG-3', MYO1B反义链: 5'-GGTCAAAGCGCT TCTTGAGG-3'; GAPDH正义链: 5'-GGACCTGACC TGCCGTCTAG-3', GAPDH反义链: 5'-GTAGCCCA GGATGCCCTTGA-3'。

1.8 Western Blot验证差异表达基因

使用含1 mmol/L PMSF的RIPA裂解液提取HOK细胞总蛋白, BCA试剂盒测蛋白浓度。7.5%的SDS-PAGE凝胶电泳分离, 转移至PVDF膜, 封闭液室温下封闭1 h, 一抗孵育, 4℃过夜, 洗膜后加

二抗, 室温孵育1 h, 增强型化学法显色, ImageQuant LAS 4000 mini显影。

1.9 统计学分析

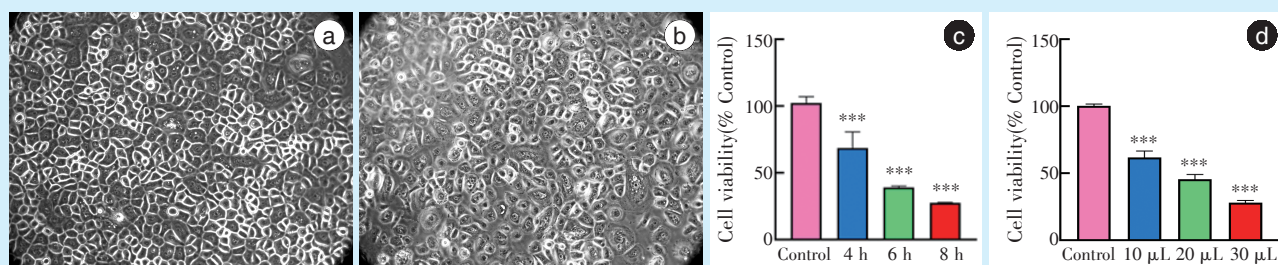
应用GraphPadPrism 8.0对数据进行统计学分析并绘制统计图。多组数据间比较采用单因素方差分析(one-way analysis of variance, ANOVA), 以Tukey进行两两比较, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 *P.m*对HOK细胞活力的影响

在倒置相差显微镜下可观察到完整的HOK单层细胞(图1a), HOK细胞与*P.m*共培养后, 细胞密度降低, 对培养皿底的黏附能力降低; 部分细胞形态发生变化, 由原本大而扁平的多角形态转变为扁圆形或圆形, 部分细胞的细胞核发生了核固缩, 显示出空泡变性和细胞裂解, 细胞边界不清晰(图1b, 10 μ L菌液与HOK细胞共培养8 h后镜下图)。

对共培养后HOK细胞的细胞活力检测结果(图1c、1d)表明, *P.m*会降低HOK细胞活力, 在时间梯度组中, 共培养4 h后细胞活力降低到 $68.38\% \pm 12.30\%$ ($P < 0.001$), 共培养6 h后细胞活力降低至 $38.97\% \pm 1.05\%$ ($P < 0.001$), 共培养8 h后细胞活力降低至 $27.44\% \pm 0.30\%$ ($P < 0.001$); 在浓度梯度组中10 μ L组细胞活力下降至 $60.33\% \pm 3.93\%$ ($P < 0.001$), 20 μ L组细胞活力下降至 $44.9\% \pm 2.77\%$ ($P < 0.001$), 30 μ L组细胞活力下降至



a: HOK cells before co-cultured with *P.m* ($\times 200$); b: HOK cells after co-cultured with *P.m* for 8 h (bacterial concentration: 5×10^6 CFU/mL)($\times 200$); c: cell viability of HOK cells after co-cultured with *P.m* in a time gradient, $F = 876.2$, $P < 0.001$; d: cell viability of HOK cells after co-cultured with *P.m* in a concentration gradient, $F = 679.7$, $P < 0.001$. *P.m*: *Prevotella melaninogenica*. HOK: human oral keratinocyte. Control: HOK and DMEM only. 4 h: *P.m* 4 h co-cultured group (bacterial concentration: 5×10^6 CFU/mL); 6 h: *P.m* 6 h co-cultured group (bacterial concentration: 5×10^6 CFU/mL); 8 h: *P.m* 8 h co-cultured group (bacterial concentration: 5×10^6 CFU/mL); 10 μ L: 10 μ L *P.m* co-cultured group (bacterial concentration: 5×10^6 CFU/mL); 20 μ L: 20 μ L *P.m* co-cultured group (bacterial concentration: 10×10^6 CFU/mL); 30 μ L: 30 μ L *P.m* co-cultured group (bacterial concentration: 15×10^6 CFU/mL). ***, $P < 0.001$

Figure 1 Cell viability of HOK cells decreased after co-cultured with *P.m*

图1 *P.m*与HOK共培养后HOK细胞活力降低

29.02% ± 2.46% ($P < 0.001$)。

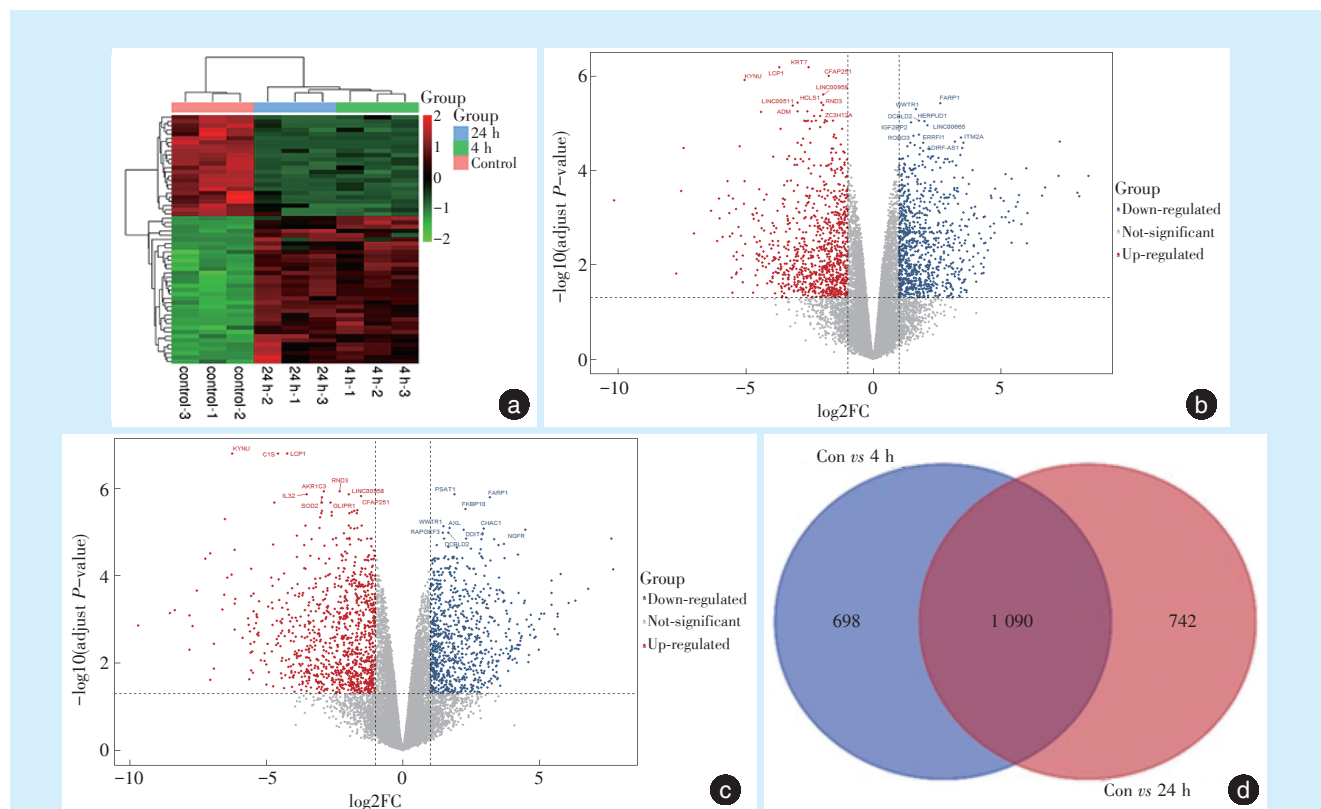
2.2 *P.m* 共培养后 pHOK 细胞差异表达基因及聚类分析

聚类分析结果显示,对照组与实验组的基因簇明显分开,提示对照组和实验组间在整体基因表达上存在明显差异(图2a)。在本次测序所获取的全部基因中,pHOK与*P.m*共培养4 h组与对照组间有1 788个基因的表达存在统计学差异,共培养24 h组与对照组间有1 832个基因的表达存在统计学差异。

pHOK与*P.m*共培养4 h组相较对照组中表达上调基因为877个,表达下调基因为916个;共培养24 h组相较对照组中表达上调基因为1 012个,表达下调基因为828个,两处理组与对照组间有1 090个共同差异表达基因(common differentially expressed genes, cDEGs)(图2b~2d,表1)。

2.3 *P.m* 共培养后 pHOK 细胞差异表达基因 GO 富集分析

对各组上调的DEGs和下调的DEGs进行GO富集分析结果显示:①对照组与共培养4 h组之间得到的上调DEGs富集于604个生物过程(biological process, BP)条目中,如细胞对脂多糖的反应(cellular response to lipopolysaccharide)、对细菌来源分子的反应(response to molecule of bacterial origin)、生物刺激的细胞反应(cellular response to biotic stimulus)等;富集于12个细胞成分(cellular component, CC)条目中,如细胞顶端部分(apical part of cell)、收缩纤维(contractile fiber)、肌节(sarcomere)等;富集于18个分子功能(molecular function, MF)条目中,如细胞因子活性(cytokine activity)、受体配体活性(receptor ligand activity)、信号受体激活因子活性(signaling receptor activator activity)等(图3a)。



a: hierarchical clustering analysis showed that gene expression profiles were different between control and *P.m* co-cultured groups, red represent upregulated genes, and green represent downregulated genes; b: volcano plot of 4 h *P.m* co-cultured group, red represent upregulated genes, and blue represent downregulated genes ($FC \geq 2.0$, $P < 0.05$); c: volcano plot of 24 h *P.m* co-cultured group; d: Venn plot of two co-cultured groups, there were 1 090 common differentially expressed genes between the two co-cultured groups and the control group; Con: control group, 4 h: *P.m* 4 h co-cultured group, 24 h: *P.m* 24 h co-cultured group. FC: fold change. *P.m*: *Prevotella melaninogenica*. pHOK: primary human oral keratinocyte. DEGs: differentially expressed genes

Figure 2 Cluster analysis and DEGs of pHOK cells co-cultured with *P.m*

图2 *P.m*与pHOK共培养后pHOK细胞聚类分析及差异表达基因情况

表1 pHOK与*P.m*共培养组较对照组上调、下调最显著的10个基因及最显著的10个共同差异表达基因Table 1 The 10 most significantly up-regulated genes and the 10 most significantly down-regulated genes in each co-cultured group and the 10 most significantly common differentially expressed genes between pHOK and *P.m* co-cultured groups and control groups

4 h up-regulated top 10	4 h down-regulated top 10	24 h up-regulated top 10	24 h down-regulated top 10	cDEGs top 10
KRT7	FARP1	KYNU	PSAT1	LCPI
LCPI	WWTR1	C1S	FARP1	KYNU
CFAP251	DCBLD2	LCPI	FKBP10	LINC00958
KYNU	HERPUD1	AKR1C3	WWTR1	CFAP251
LINC00958	IGF2BP2	RND3	AXL	RND3
HCLS1	LINC00665	IL-32	CHAC1	C1S
LINC00511	ERRFI1	LINC00958	NGFR	FARP1
RND3	ROBO3	CFAP251	DDIT4	KRT7
ADM	ITM2A	SOD2	RAPGEF3	LINC00511
ZC3H12A	TSSC2	HEPHL1	DCBLD2	SOD2

Co-culture groups: pHOK and *P.m* co-culture; control groups: pHOK and DMEM only. *P.m*: *Prevotella melaninogenica*. pHOK: primary human oral keratinocyte. cDEGs: common differentially expressed genes. KRT7: keratin 7; LCPI: lymphocyte cytosolic protein 1; CFAP251: cilia and flagella associated protein 251; KYNU: kynureninase; LINC00958: long intergenic non-protein coding RNA 958; HCLS1: hematopoietic cell-specific lyn substrate 1; LINC00511: long intergenic non-protein coding RNA 511; RND3: Rho family GTPase 3; ADM: adrenomedullin; ZC3H12A: zinc finger CCCH-type containing 12A; FARP1: FERM, ARH/RhoGEF and pleckstrin domain protein 1; WWTR1: WW domain containing transcription regulator 1; DCBLD2: discoidin, CUB and LCCL domain containing 2; HERPUD1: homocysteine inducible ER protein with ubiquitin like domain 1; IGF2BP2: insulin like growth factor 2 mRNA binding protein 2; LINC00665: long intergenic non-protein coding RNA 665; ERRFI1: ERBB receptor feedback inhibitor 1; ROBO3: roundabout guidance receptor 3; ITM2A: integral membrane protein 2A; TSSC2: tumor suppressing subtransferable candidate 2; C1S: complement C1s; AKR1C3: Aldo-Keto reductase family 1 member C3; IL-32: interleukin-32; SOD2: superoxide dismutase 2; HEPHL1: hephaestin like 1; PSAT1: phosphoserine aminotransferase 1; FKBP10: FKBP prolyl isomerase 10; AXL: AXL receptor tyrosine kinase; CHAC1: ChaC glutathione specific gamma-glutamyl-cyclotransferase 1; NGFR: nerve growth factor receptor; DDIT4: DNA damage inducible transcript 4; RAPGEF3: Rap guanine nucleotide exchange factor 3; DCBLD2: discoidin, CUB and LCCL domain containing 2

②对照组与共培养4 h组之间得到的下调DEGs富集于19个生物过程条目中,如上皮发育(epidemis development)、皮肤发育(skin development)、上皮细胞分化调节(regulation of epithelial cell differentiation)等;富集于1个细胞成份条目中,即角质化包膜(cornified envelope)(图3b)。

③对照组与共培养24 h组之间得到的上调DEGs富集的GO通路与4 h组在分子功能富集条目相似度高(图3c)。

④对照组与共培养24 h组之间得到的下调DEGs富集于84个生物过程条目中,如化学突触传递的调节(modulation of chemical synaptic transmission)、跨突触信号的调节(regulation of trans-synaptic signaling)、神经递质转运(neurotransmitter transport)等;富集于21个细胞成份条目中,如内质网腔(endoplasmic reticulum lumen)、神经元与神经元突触(neuron to neuron synapse)、突触后密集区(post-synaptic density)等;在MF条目中没有具有统计学差异的富集(图3d)。

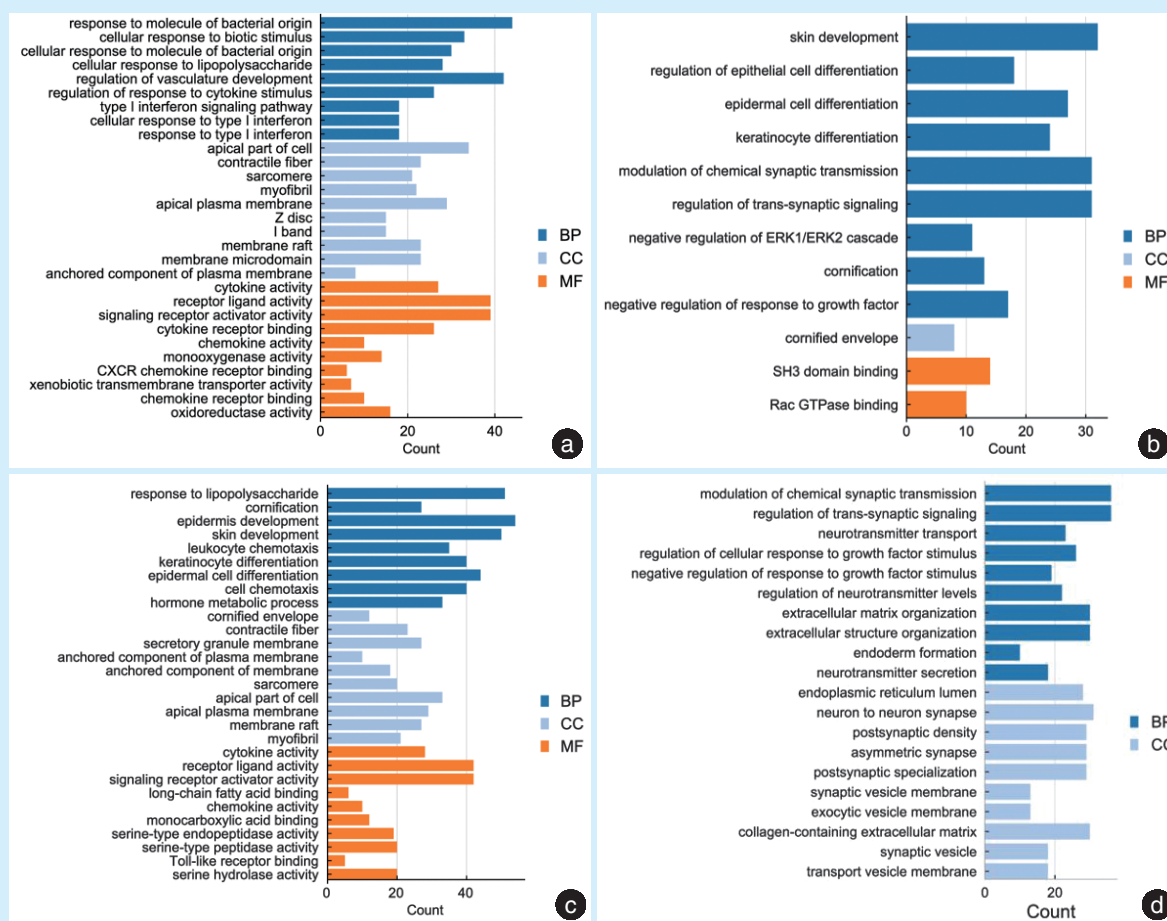
2.4 *P.m*共培养后pHOK细胞差异表达基因KEGG通路富集分析

①对照组与共培养4 h组之间得到的上调DEGs富集于33条KEGG通路中,显示其与细胞因子与细胞因子受体相互作用(cytokine-cytokine receptor interaction)、病毒蛋白与细胞因子和细胞因子受体相互作用(viral protein interaction with cytokine and cytokine receptor)、肿瘤坏死因子(tumor necrosis factor, TNF)信号通路等相关(图4a)。

②对照组与共培养4 h组之间得到的下调DEGs在KEGG通路数据库中没有得到具有统计学差异的富集条目。

③对照组与共培养24 h组之间得到的上调DEGs富集于12条KEGG通路中,显示其与白介素-17(interleukin-17, IL-17)信号通路、TNF信号通路、金黄色葡萄球菌感染(*Staphylococcus aureus* infection)等相关(图4b)。

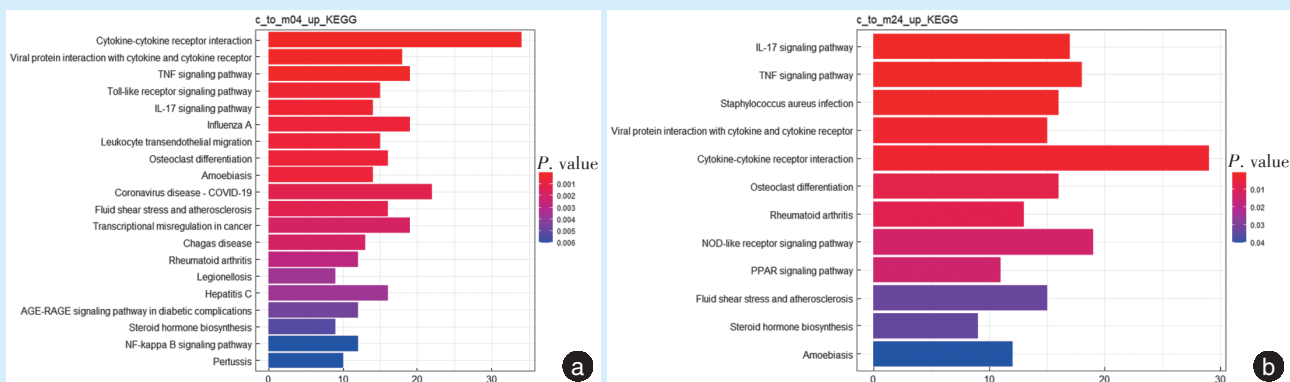
④对照组与共培养24 h组之间得到的下调DEGs在KEGG通路数据库中没有得到具有统计学差异的富集条目。



a: *P.m* 4 h co-cultured group upregulated GO pathway; b: *P.m* 4 h co-cultured group downregulated GO pathway; c: *P.m* 24 h co-cultured group upregulated GO pathway; d: *P.m* 24 h co-cultured group downregulated GO pathway. *P.m*: *Prevotella melaninogenica*. pHOK: primary human oral keratinocyte. BP: biological process. CC: cellular component. MF: molecular function. GO: gene ontology

Figure 3 Gene ontology analysis of pHOK cells co-cultured with *P.m*

图3 *P.m* 与 pHOK 共培养后 pHOK 细胞的 GO 分析



a: *P.m* 4 h co-cultured group upregulated KEGG pathway; b: *P.m* 24 h co-cultured group upregulated KEGG pathway. KEGG: Kyoto Encyclopedia of Genes and Genomes. *P.m*: *Prevotella melaninogenica*. pHOK: primary human oral keratinocyte

Figure 4 KEGG pathway analysis of pHOK cells co-cultured with *P.m*

图4 *P.m* 与 pHOK 共培养后 pHOK 细胞 KEGG 通路分析

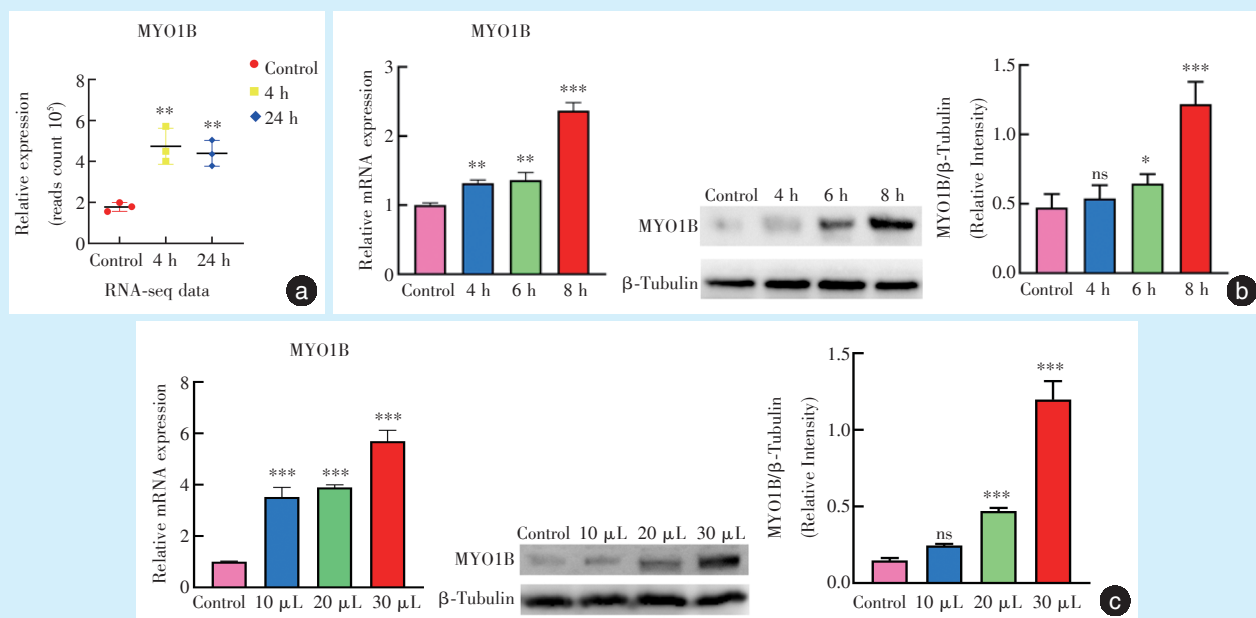
2.5 差异表达基因肌球蛋白1B的验证

在本次转录组测序结果中,MYO1B是共培养4 h组、共培养24 h组共有的DEG。

将相同浓度(5×10^6 CFU/mL)的*P.m*与HOK细胞共培养不同时长(0、4 h、6 h、8 h),MYO1B的mRNA表达和蛋白表达在4、6、8 h均显著增加,差

异有统计学意义($P < 0.005$)。

将不同浓度的*P.m*(10 μ L组、20 μ L组、30 μ L组最终菌液浓度分别为: 5×10^6 CFU/mL、 10×10^6 CFU/mL、 15×10^6 CFU/mL)刺激HOK细胞4 h后MYO1B的表达均显著增加,差异有统计学意义($P < 0.005$)(图5)。



a: expression of MYO1B in RNA-seq data, $F = 19.4$; b: MYO1B mRNA ($F = 330.2$) and protein expressions ($F = 54.25$) upregulated in HOK cells after *P.m* stimulated in time gradient; c: MYO1B mRNA ($F = 266.0$) and protein expressions ($F = 275.3$) upregulated in HOK cells after *P.m* stimulated in concentration gradient. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant. *P.m*: *Prevotella melaninogenica*. HOK: human oral keratinocyte. 4 h: *P.m* 4 h co-cultured group (bacterial concentration: 5×10^6 CFU/mL); 6 h: *P.m* 6 h co-cultured group (bacterial concentration: 5×10^6 CFU/mL); 8 h: *P.m* 8 h co-cultured group (bacterial concentration: 5×10^6 CFU/mL); 10 μ L: 10 μ L *P.m* co-cultured group (bacterial concentration: 5×10^6 CFU/mL); 20 μ L: 20 μ L *P.m* co-cultured group (bacterial concentration: 10×10^6 CFU/mL); 30 μ L: 30 μ L *P.m* co-cultured group (bacterial concentration: 15×10^6 CFU/mL). Control: HOK and DMEM only. MYO1B: myosin 1B

Figure 5 Verification of MYO1B expression in HOK cells co-cultured with *P.m*

图5 *P.m*与HOK共培养后HOK细胞MYO1B表达的验证

3 讨论

细菌微生物的菌群失调可能是OLP发生发展的原因之一。本课题组前期发现*P.m*在OLP患者颊黏膜表面构成比增加最为显著,而口腔上皮角质形成细胞作为口腔黏膜抵御外界入侵的第一道防线,可能直接受*P.m*刺激影响。在与*P.m*共培养后,HOK细胞在镜下可保持完整的细胞形态并形成完整的单层细胞,*P.m*的刺激会降低HOK细胞的活力,对HOK细胞造成损伤;在对OLP患者的口腔角质形成细胞进行体外分离培养后,发现较正常人群相比,OLP患者的口腔角质形成细胞的细胞活力明显降低,细胞形态也发生明显变化^[10],本课题

组建立的*P.m*-HOK细胞共培养模型与之相似,推测*P.m*损伤HOK细胞可能与OLP临床中发生上皮层糜烂和溃疡有关。

目前认为免疫失调在OLP发病过程中起到重要作用^[11],多个学者报道的OLP组织高通量测序结果^[12-13]显示其多富集在与免疫相关的GO与KEGG通路如趋化因子活跃、细胞因子活跃和原发性免疫缺陷等免疫炎症相关通路,以及与上皮发育分化、神经受体配体作用和代谢相关的通路如表皮分化复合体^[14]、激活神经元的受体配体交互和酪氨酸代谢^[15]等。有文献报道OLP组织中Toll样受体-4(toll like receptor-4, TLR-4)的明显高表

达^[16],且存在多种细胞因子表达改变,如肿瘤坏死因子 α (tumor necrosis factor- α , TNF- α)表达升高^[10],白介素-23(interleukin-23, IL-23)和IL-17过表达等^[17]。为探究*P.m*对pHOK短时间作用及较长时间作用的影响,并探究*P.m*刺激与OLP发生发展之间的关联,本研究进一步将*P.m*与pHOK共培养后4 h和24 h进行转录组测序,研究结果显示,*P.m*刺激pHOK细胞后,炎症相关通路如IL-17信号通路、肿瘤坏死因子信号通路、Toll样受体通路、细胞因子与细胞因子受体互作等在两处理组中均明显富集,其他KEGG通路如上皮发育、跨突触信号调节等也有较明显富集,而这些通路与既往报道的OLP转录组测序的富集通路有明显重合。

本研究结果显示,*P.m*与pHOK共培养4 h组GO通路明显富集在细胞顶端部分(apical part of cell)和顶端质膜(apical plasma membrane)这两个细胞成分中。单个上皮细胞之间的空间密封由顶端连接复合物(apical junctional complex)执行,其包含紧密连接、黏附连接和桥粒^[18]。细胞黏附建立后,紧密连接通过其蛋白质组分将空间密封起来^[19]。本课题组推测,在*P.m*刺激pHOK细胞早期,pHOK细胞的细胞顶端部分和顶端质膜的顶端连接复合物成分合成加强,以增强细胞间紧密连接,抵抗外界细菌侵入。

本研究结果显示,MYO1B是pHOK与*P.m*共培养4 h、24 h组均上调表达的差异基因。MYO1B即肌球蛋白1B,肌球蛋白是一种以ATP调节的方式结合到肌动蛋白的蛋白质,与肌动蛋白的结合促进肌球蛋白水解ATP,从而为肌动蛋白丝的运动提供动力^[20]。肌动-肌球蛋白的收缩是细胞骨架改变的形式之一,肌动蛋白-肌球蛋白环也是组成细胞紧密连接的重要部分,肌动蛋白-肌球蛋白环在维持上皮极性和上皮屏障功能中发挥了重要作用^[21]。Lv等^[22]报道,肌球蛋白II A(myosin-II A)调控缺氧低糖脑内皮细胞的紧密连接,抑制肌球蛋白II A可减弱claudin-5和ZO-1等紧密连接蛋白的形态变化;Omelchenko等^[23]报道,在整体迁移的细胞中,肌球蛋白IX A(myosin-IX A)的敲除导致肌动蛋白细胞骨架的改变,细胞-细胞间黏附被破坏,导致细胞分散。以上这些实验表明,肌球蛋白家族通过细胞骨架与紧密连接调节了上皮细胞屏障。本研究结果显示,*P.m*浓度梯度对MYO1B表达的影响更为明显,提示菌群数量的失衡对紧密连接有较大影响。这与本课题组前期研究发现在OLP

患者颊黏膜表面菌群失调相印证。此外,文献报道MYO1B参与了舌部鳞状细胞癌等多种肿瘤的发展与转移,且预示了较差的预后以及隐匿性淋巴结转移^[24]。提示MYO1B可能参与了OLP恶变。

综上,本研究对与*P.m*共培养4 h和24 h的pHOK细胞进行转录组测序,研究发现*P.m*和pHOK共培养过程中IL-17信号通路、肿瘤坏死因子信号通路、Toll样受体通路等KEGG通路明显富集,对脂多糖的反应、对细菌来源分子的反应、细胞顶端部分等GO通路明显富集,提示*P.m*对口腔角质细胞的转录组存在重要影响,其中MYO1B可能在细菌-细胞相互作用中发挥了重要作用。本研究为进一步揭示OLP的发病机制提供了相关的研究基础。

【Author contributions】 Guo YT performed the experiments and wrote the article. Han WH analyzed the data. Xu P, Shao RR assisted in the experiments. He Y designed the study and reviewed the article. All authors read and approved the final manuscript as submitted.

参考文献

- [1] Li C, Tang X, Zheng X, et al. Global prevalence and incidence estimates of oral lichen planus: a systematic review and meta-analysis[J]. JAMA Dermatol, 2020, 156(2): 172-181. doi: 10.1001/jama-dermatol.2019.3797.
- [2] Alexander KL, Targan SR, Elson CO. Microbiota activation and regulation of innate and adaptive immunity[J]. Immunol Rev, 2014, 260(1): 206-220. doi: 10.1111/imr.12180.
- [3] Li Y, Wang K, Zhang B, et al. Salivary mycobiome dysbiosis and its potential impact on bacteriome shifts and host immunity in oral lichen planus[J]. Int J Oral Sci, 2019, 11(2): 1-10. doi: 10.1038/s41368-019-0045-2.
- [4] Wei W, Sun Q, Deng Y, et al. Mixed and inhomogeneous expression profile of Th1/Th2 related cytokines detected by cytometric bead array in the saliva of patients with oral lichen planus[J]. Oral Surg Oral Med Oral Pathol Oral Radiol, 2018, 126(2): 142-151. doi: 10.1016/j.oooo.2018.02.013.
- [5] Zheng S, Xu P, Cai L, et al. The presence of *Prevotella melaninogenica* within tissue and preliminary study on its role in the pathogenesis of oral lichen planus[J]. Oral Dis, 2021. doi: 10.1111/odi.13862.
- [6] Lin D, Yang L, Wen L, et al. Crosstalk between the oral microbiota, mucosal immunity, and the epithelial barrier regulates oral mucosal disease pathogenesis[J]. Mucosal Immunol, 2021, 14(6): 1247-1258. doi: 10.1038/s41385-021-00413-7.
- [7] Yang QZ, Guo B, Sun HY, et al. Identification of the key genes implicated in the transformation of OLP to OSCC using RNA-seq[J]. Oncol Rep, 2017, 37(4): 2355-2365. doi: 10.3892/or.2017.5487.
- [8] Vo PT, Choi SS, Park HR, et al. Gene signatures associated with

- barrier dysfunction and infection in oral lichen planus identified by analysis of transcriptomic data[J]. *PLoS One*, 2021, 16(9): e0257356. doi: 10.1371/journal.pone.0257356.
- [9] 张羽婷, 刘江, 赵行, 等. 减毒5-氟尿嘧啶乳糖苷衍生物的合成及抗口腔鳞状细胞癌活性的实验研究[J]. *华西口腔医学杂志*, 2022, 40(1): 32-38. doi: 10.7518/hxkq.2022.01.005.
- Zhang YT, Liu J, Zhao H, et al. Synthesis of 5-fluorouracil-lactoside derivatives and experimental study on their anti-oral squamous cell carcinoma activity[J]. *West Chin J Stomatol*, 2022, 40(1): 32-38. doi: 10.7518/hxkq.2022.01.005.
- [10] Cao T, Zhang H, Zhou L, et al. *In vitro* cell culture system optimization of keratinocytes from oral lichen planus (OLP) patients[J]. *Oral Dis*, 2017, 23(2): 225-232. doi: 10.1111/odi.12599.
- [11] Lodi G, Scully C, Carrozzo M, et al. Current controversies in oral lichen planus: report of an international consensus meeting. Part 1. Viral infections and etiopathogenesis[J]. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2005, 100(1): 40-51. doi: 10.1016/j.tripleo.2004.06.077.
- [12] Gassling V, Hampe J, Açil Y, et al. Disease-associated miRNA-mRNA networks in oral lichen planus[J]. *PLOS ONE*, 2013, 8(5): e63015. doi: 10.1371/journal.pone.0063015.
- [13] Chen J, Du G, Wang Y, et al. Integrative analysis of mRNA and miRNA expression profiles in oral lichen planus: preliminary results[J]. *Oral Surg Oral Med Oral Pathol Oral Radiol*, 2017, 124(4): 390-402. doi: 10.1016/j.oooo.2017.05.513.
- [14] Danielsson K, Coates P, Ebrahimi M, et al. Genes involved in epithelial differentiation and development are differentially expressed in oral and genital lichen planus epithelium compared to normal epithelium[J]. *Acta Derm Venereol*, 2014, 94(5): 526-530. doi: 10.2340/00015555-1803.
- [15] Liu Q, Wang X, Liu Y, et al. A combinative analysis of gene expression profiles and microRNA expression profiles identifies critical genes and microRNAs in oral lichen planus[J]. *Arch Oral Biol*, 2016, 68: 61-65. doi: 10.1016/j.archoralbio.2016.03.018.
- [16] Janardhanam SB, Prakasam S, Swaminathan VT, et al. Differential expression of TLR-2 and TLR-4 in the epithelial cells in oral lichen planus[J]. *Arch Oral Biol*, 2012, 57(5): 495 - 502. doi: 10.1016/j.archoralbio.2011.10.013.
- [17] Lu R, Zeng X, Han Q, et al. Overexpression and selectively regulatory roles of IL-23/IL-17 axis in the lesions of oral lichen planus[J]. *Mediators Inflamm*, 2014: 701094. doi: 10.1155/2014/701094.
- [18] Hermiston ML, Gordon JL. *In vivo* analysis of cadherin function in the mouse intestinal epithelium: essential roles in adhesion, maintenance of differentiation, and regulation of programmed cell death[J]. *J Cell Biol*, 1995, 129(2): 489 - 506. doi: 10.1083/jcb.129.2.489.
- [19] González-Mariscal L, Betanzos A, Nava P, et al. Tight junction proteins[J]. *Prog Biophys Mol Biol*, 2003, 81(1): 1 - 44. doi: 10.1016/S0079-6107(02)00037-8.
- [20] Woolner S, Bement WM. Unconventional myosins acting unconventionally[J]. *Trends Cell Biol*, 2009, 19(6): 245 - 252. doi: 10.1016/j.tcb.2009.03.003.
- [21] Campos Y, Qiu X, Gomero E, et al. Alix-mediated assembly of the actomyosin-tight junction polarity complex preserves epithelial polarity and epithelial barrier[J]. *Nat Commun*, 2016, 7(1): 11876. doi: 10.1038/ncomms11876.
- [22] Lv Y, Liu W, Ruan Z, et al. Myosin II A regulated tight junction in oxygen glucose-deprived brain endothelial cells *via* activation of TLR4/PI3K/Akt/JNK1/2/14-3-3 ϵ /NF- κ B/MMP9 signal transduction pathway[J]. *Cell Mol Neurobiol*, 2019, 39(2): 301 - 319. doi: 10.1007/s10571-019-00654-y.
- [23] Omelchenko T, Hall A. Myosin-IXA regulates collective epithelial cell migration by targeting RhoGAP activity to cell-cell junctions[J]. *Curr Biol*, 2012, 22(4): 278-288. doi: 10.1016/j.cub.2012.01.014.
- [24] Thangaraj SV, Shyamsundar V, Krishnamurthy A, et al. Deregulation of extracellular matrix modeling with molecular prognostic markers revealed by transcriptome sequencing and validations in oral tongue squamous cell carcinoma[J]. *Sci Rep*, 2021, 11(1): 250. doi: 10.1038/s41598-020-78624-4.

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