



IL-17信号通路在高原低氧诱导小鼠脾脏炎症反应的作用机制*

永胜, 郭玉静, 陈晓晨, 许玉珍, 胡英[△]

青海大学医学院基础医学部免疫学教研室(西宁810016)

【摘要】目的 探究高原低氧环境诱导小鼠脾脏组织炎症反应发生的作用机制。**方法** 将C57BL/6小鼠随机分为平原常氧组和高原低氧组,每组5只。其中,平原常氧组饲养于海拔400 m(氧浓度19.88%)常氧浓度环境,高原低氧组小鼠置于海拔4200 m(氧浓度14.23%)高原环境,构建高原低氧动物模型。第30天取脾脏组织检测脾脏指数,HE染色观察小鼠脾脏组织病理学改变,实时荧光定量PCR(RT-qPCR)和Western blot检测小鼠脾脏组织白细胞介素(IL)-6、IL-12、IL-1 β 的mRNA和蛋白表达量;使用RNA测序(RNA-sequencing, RNA-seq)技术进行高通量转录组学测序,将差异表达基因(differentially expressed genes, DEGs)进行KEGG富集分析,对关键通路中的DEGs进行RT-qPCR验证。**结果** 与平原常氧组相比,高原低氧环境下,小鼠脾脏指数降低($P<0.05$),并出现白髓减少,生发中心扩大,边缘模糊,静脉充血等病理学改变;高原低氧组小鼠脾脏组织IL-6、IL-12、IL-1 β 的mRNA和蛋白表达量均上调($P<0.05$)。经转录组测序,KEGG通路富集分析示,4218个DEGs共富集到178个富集通路($P<0.05$),DEGs在T细胞受体信号通路、TNF信号通路和IL-17信号通路等多个与免疫、炎症相关通路中显著富集($P<0.05$);其中,IL-17信号通路和下游炎症因子高度上调表达($P<0.05$);与平原常氧组相比,高原低氧组小鼠脾脏组织IL-17信号通路中关键基因IL-17、IL-17R、MAPKs(mitogen-activated protein kinase genes)等和下游炎症因子MMP9(matrix metalloproteinase 9)、S100A8(S100 calcium binding protein A8 gene)、S100A9(S100 calcium binding protein A9 gene)、肿瘤坏死因子- α (tumour necrosis factor α , TNF- α)等基因的mRNA表达量均出现上调或下调差异表达($P<0.05$)。经RT-qPCR验证,DEGs的mRNA表达量与RNA-seq结果一致。**结论** 高原低氧环境可通过激活IL-17信号通路,促进下游炎症因子释放,诱导小鼠脾脏组织炎症反应发生。

【关键词】 高原低氧 转录组学 IL-17信号通路 脾脏组织 炎症 RNA测序 生物信息学

Mechanism of IL-17 Signaling Pathway in Spleen Inflammatory Response Induced by Altitude Hypoxia in Mice

YONG Sheng, GUO Yujing, CHEN Xiaochen, XU Yuzhen, HU Ying[△]. Department of Immunology, School of Basic Medicine, Qinghai University School of Medical, Xining 810016, China

[△] Corresponding author, E-mail: hyyx-66@163.com

【Abstract】 Objective To explore the mechanism of spleen tissue inflammatory response induced by altitude hypoxia in mice. **Methods** C57BL/6 mice were randomly assigned to a plain, i.e., low-altitude, normoxia group and an altitude hypoxia group, with 5 mice in each group. In the plain normoxia group, the mice were kept in a normoxic environment at the altitude of 400 m above sea level (with an oxygen concentration of 19.88%). The mice in the altitude hypoxia group were kept in an environment at the altitude of 4200 m above sea level (with an oxygen concentration of 14.23%) to establish the animal model of altitude hypoxia. On day 30, spleen tissues were collected to determine the splenic index. HE staining was performed to observe the histopathological changes in the spleen tissues of the mice. Real time fluorogenic quantitative PCR (RT-qPCR) and Western blot were conducted to determine the mRNA and protein expressions of interleukin (IL)-6, IL-12, and IL-1 β in the spleen tissue of the mice. High-throughput transcriptome sequencing was performed with RNA sequencing (RNA-seq). KEGG enrichment analysis was performed for the differentially expressed genes (DEGs). The DEGs in the key pathways were verified by RT-qPCR. **Results** Compared with the plain normoxia group, the mice exposed to high-altitude hypoxic environment had decreased spleen index ($P<0.05$) and exhibited such pathological changes as decreased white pulp, enlarged germinal center, blurred edge, and venous congestion. The mRNA and protein expression levels of IL-6, IL-12, and IL-1 β in the spleen tissue of mice in the altitude hypoxia group were up-regulated ($P<0.05$). According to the results of transcriptome sequencing and KEGG pathway enrichment analysis, 4218 DEGs were enriched in 178 enrichment pathways ($P<0.05$). DEGs were significantly enriched in multiple pathways associated with immunity and inflammation, such as T cell receptor signaling pathway, TNF signaling pathway, and IL-17 signaling pathway ($P<0.05$) in the spleen of mice exposed to high-altitude hypoxic environment. Among them, IL-17 signaling pathway and the downstream inflammatory factors were highly up-regulated ($P<0.05$). Compared with the plain normoxia group, the mRNA expression levels of key genes in the IL-17 signaling pathway, including IL-17, IL-17R, and mitogen-activated protein kinase genes (MAPKs), and the downstream

* 国家自然科学基金(No. 82060295)、青海省科技厅科技计划项目基金(No. 2023-ZJ-771)和青海大学青年科研基金(No. 2021-QNY-10)资助

[△] 通信作者, E-mail: hyyx-66@163.com

出版日期: 2024-01-20

inflammatory factors, including matrix metalloproteinase 9 (MMP9), S100 calcium binding protein A8 gene (S100A8), S100 calcium binding protein A9 gene (S100A9), and tumor necrosis factor α (TNF- α), were up-regulated or down-regulated ($P < 0.05$) in the altitude hypoxia group. According to the validation of RT-qPCR results, the mRNA expression levels of DEGs were consistent with the RNA-seq results. **Conclusion** Altitude hypoxia can induce inflammatory response in the mouse spleen tissue by activating IL-17 signaling pathway and promoting the release of downstream inflammatory factors.

【Key words】 Altitude hypoxia Transcriptomics IL-17 signaling pathway Spleen tissue
Inflammation RNA-sequencing Bioinformatics

高原低氧环境是诱导组织和细胞炎症反应发生的主要因素之一。研究发现,急性低压低氧会促进小鼠体内脂多糖产生,从而诱导全身炎症反应^[1]。模拟高原低氧环境,小鼠脾脏总抗氧化能力、过氧化物歧化酶的活性均下降,且小鼠脾脏炎性细胞数量均明显升高^[2]。脾脏是机体最大的外周免疫器官,是免疫细胞定居和发生免疫应答及体液调节的重要场所^[3]。当炎症反应发生时,免疫细胞受到组织环境中的细胞因子或微生物肽等刺激后,其代谢状态会发生不同类型的改变,如招募活化的中性粒细胞在炎症部位聚集,活化巨噬细胞和淋巴细胞增加糖酵解的速率等^[4-5]。因此,高原低氧环境对小鼠脾脏组织炎症反应的发生机制亟待引起广泛重视。随着高通量测序技术的快速发展,基于RNA测序(RNA sequencing, RNA-seq)的转录组分析技术,在探究高原低氧环境对各组织系统的响应机制中应用越来越广泛^[6-7]。因此,本研究通过RNA-seq技术,探究高原低氧环境诱导小鼠脾脏组织炎症反应发生的作用机制,挖掘与炎症反应相关的关键通路并明确该通路在高原低氧环境下的影响机制。

1 材料与方法

1.1 主要试剂与仪器

Trizol裂解液(Invitrogen公司,美国);逆转录试剂盒和实时荧光定量PCR(qPCR)试剂盒(TaKaRa公司,日本);引物(生工生物有限公司,中国上海);硝酸纤维素(NC)膜(Pall Corporation公司,美国);兔源缺氧诱导因子1 α (HIF-1 α)蛋白抗体、兔源白细胞介素-6(IL-6)蛋白抗体、兔源白细胞介素-1 β (IL-1 β)蛋白抗体和兔源白细胞介素-12(IL-12)蛋白抗体(Affinity公司,美国);辣根过氧化物酶标记的山羊抗兔二抗(Proteintech公司,美国);增强化学发光液(Thermo Fisher Scientific公司,美国)。荧光定量PCR仪(Roche公司,瑞士);电泳仪、转膜仪(Bio-Rad公司,美国);凝胶成像系统(VILBER公司,法国)。

1.2 实验动物

参考既往文献^[8-9],实验选用6~8周龄C57BL/6雄性健康小鼠,体质量(18 \pm 2)g,购自西安交通大学医学部实验

动物中心,动物生产许可证号:SYXK(陕)2020-005。将小鼠随机分为平原常氧(plain spleen control, PSC)组和高原低氧(hypoxic spleen test, HST)组,每组5只。其中,PSC组饲养于海拔400 m(氧浓度19.88%)的西安交通大学医学部实验动物中心,HST组饲养于海拔4 200 m(氧浓度14.23%)的青海省果洛藏族自治州玛多县人民医院实验动物房。期间保证温度、湿度及饮水、进食一致的饲养条件。30 d后无菌采集小鼠脾脏,一部分液氮保存备用,另一部分置于体积分数4%多聚甲醛中固定保存。该项目已通过青海大学伦理协会审查(批准号:2020-15)。

1.3 小鼠脾脏指数测定和脾脏组织病理学HE染色

称量小鼠空腹体质量后,无菌取出小鼠脾脏组织进行称量。通过以下公式计算小鼠脾脏指数:脾脏指数=脾脏质量/体质量 \times 100%。质量单位均为g。

将小鼠脾脏组织置于体积分数4%多聚甲醛固定,梯度乙醇脱水处理,随后将组织石蜡包埋切片5~6 μ m,二甲苯脱蜡后,使用常规HE染色,显微镜下观察组织切片。

1.4 实时荧光定量PCR (RT-qPCR)

使用TRIzol法提取小鼠脾脏组织总RNA,进行RNA浓度及纯度测定,并立即储存于-80 $^{\circ}$ C冰箱。使用分光光度计进行测量RNA浓度及260/280、260/230吸光度比值。随后,通过Agilent 2100 bioanalyzer精确检测RNA完整性,对RNA样品进行严格质控。将RNA逆转录为cDNA。反应体系为TB Green[®] Premix Ex Taq[™] II 10 μ L, dH₂O 6.4 μ L, cDNA 2 μ L,上下游引物各0.8 μ L。RT-qPCR反应条件为95 $^{\circ}$ C预变性30 s;95 $^{\circ}$ C 5 s,63 $^{\circ}$ C 60 s,循环40次扩增;95 $^{\circ}$ C 10 s,65 $^{\circ}$ C 60 s,97 $^{\circ}$ C 1 s溶解;37 $^{\circ}$ C冷却30 s。以 β -actin为内参,采用 $2^{-\Delta\Delta Ct}$ 法计算相关基因mRNA相对表达量,引物序列详见表1。

1.5 蛋白免疫印迹 (Western blot)

提取小鼠脾脏组织蛋白,并使用BCA法对所提取蛋白进行浓度测定,调整上样浓度为30 μ g/孔。随后经SDS-PAGE凝胶电泳、转膜、5%脱脂牛奶封闭、洗膜、加入一抗IL-6(1:500)、IL-12(1:500)、IL-1 β (1:500)、内参 β -actin(1:10 000)4 $^{\circ}$ C孵育过夜。加入羊抗兔二抗(1:

表 1 RT-qPCR引物信息
Table 1 RT-qPCR primer information

Gene	Primer sequence (5'-3')	Product length/bp
β -actin	F: CATCCGTAAGACCTCTATGCCAAC	25
	R: ATGGAGCCACCGATCCACA	19
IL-6	F: CCACCTCACAAGTCGGAGGCTTA	23
	R: TGCAAGTGCATCATAGTTGTTTC	22
IL-12	F: GGAGCAGCTGTTGATGGACCTA	22
	R: AATCCAGAACATGCCGACA	20
IL-1 β	F: TCCAGGATGAGGACATGAGCAC	22
	R: GAACGTCACACACCAGCAGGTTA	23
IL-17	F: AACTCCGTCGCCATTCAGCAAG	22
	R: ACACAGGTGCAGCCAACTTTTAGG	24
IL-17RA	F: TGAGCTGCAGAGTCAAGAATAG	22
	R: CATGCAACACAGGGACTAATTC	22
IL-17RC	F: GCTTCTTACTGTACTCTGCGTCCAG	24
	R: CTCTAGCGACCACACCTGAATGC	23
ACT1	F: CCAGAGCATCGGTTACACTTACG	24
	R: CCACTGAAGGAGTCTGCCAAAGC	23
TRAF6	F: GAAAATCAACTGTTCCCGACA	22
	R: ACTTGATGATCCTCGAGCTGTC	22
TRAF3	F: GATCTGGAAGATCCGTGACTAC	22
	R: TCCATCAGCATAAGTGTCACTT	22
TRAF4	F: CGAAGAGAGCTGGAAGAATAT	22
	R: TTGTAGCCGTAATATGCGTAT	22
MAPK8	F: TTGAAAACAGGCCTAAATACGC	22
	R: GTTTGTATGCTCTGAGTCAGC	22
MAPK9	F: GCAGGTGGCGGACTCAACTTTC	22
	R: AAAGCAGCACAAACAATTCCTGGG	25
MAPK13	F: CAGCCTTTTGATGATGCCTTAG	22
	R: TGGGACTGAAGTTTGAGATCTC	22
TAB2	F: ATTTCAACAGGACCTCGAAAGA	22
	R: GGTGGCATTGGCTGAAATATAG	23
TAB3	F: GCAGCACCAGAACCTATT	18
	R: TCTTGCTCGCTGATGTAA	18
RELA	F: AGACCCAGGAGTGTTCACAGACC	23
	R: GTCACCAGGCGAGTTATAGCTTCAG	25
MMP9	F: CAAAGACTGAAAACCTCCAAC	22
	R: GACTGCTTCTCTCCATCATC	21
S100A8	F: CTTGAGCAACCTCATTGATGTC	22
	R: GGAACCTCTCGAAGTTAATTGC	22
S100A9	F: GGAAGCACAGTTGGCAACCTTTA	23
	R: GATCAACTTGGCCATCAGCATCA	23
LCN2	F: CGCTACTGGATCAGAACATTG	22
	R: CTTGCACATTGTAGCTCTGTAC	22
TNF- α	F: ATGTCTCAGCCTCTTCTCATT	22
	R: GCTTGTCACTCGAATTTTGAGA	22
CXCL5	F: TGATCGCTAATTTGGAGGTGAT	22
	R: TAGCTTTCTTTTGTCACTGCC	22

IL: interleukin; IL-17RA: interleukin 17 receptor A; IL-17RC: interleukin 17 receptor C; ACT1: actin related gene 1; TRAF: TNF receptor-associated factor; MAPK: mitogen-activated protein kinase gene; TAB: TGF- β activated kinase 1/MAP3K7 binding protein gene; RELA: v-rel reticuloendotheliosis viral oncogene homolog A (avian); MMP9: matrix metalloproteinase 9; S100A8: S100 calcium binding protein A8 gene; S100A9: S100 calcium binding protein A9 gene; LCN2: lipocalin 2; TNF- α : tumour necrosis factor α ; CXCL5: C-X-C motif chemokine ligand 5.

5 000), 室温孵育 1 h。洗膜, 用 ECL 化学发光液进行显色, 凝胶成像系统观察分析灰度值。以目的条带灰度值与内参条带灰度值的比值, 为目的蛋白的相对表达量。

1.6 使用 RNA-seq 技术进行高通量转录组学测序

去除 RNA 样本中的核糖体 RNA 获得 mRNA, 随机将各组 mRNA 剪成片段, 反转录为 cDNA。在平末端和磷酸化的 cDNA 中添加一个 3' 腺苷片段和修复末端的 Illumina 接头。PCR 扩增文库, 纯化文库, 将扩增的文库通过 Illumina 测序平台进行测序 (北京诺禾致源有限公司)。对原始数据 (raw reads) 进行质量控制。进一步对转录组学测序结果进行主成分分析 (PCA) 后发现, 组间样本分散, 组内样本聚集, 提示样本离散程度好。随后, 参考文献方法^[10]对转录组学测序结果进行验证, 发现本次转录组测序结果可靠, 可用于后续生物信息学分析。

1.7 生物信息学分析

使用 FeatureCounts 进行基因原始表达量的统计。为了消除样本测序数据量、基因序列长度等差异引起的基因表达量统计偏差, 采用 FPKM 方法对样本基因表达量进行标准化。使用 R 工具包 DESeq2 分析组间差异表达基因 (differentially expressed genes, DEGs), 并采用 B&H 方法 (Benjamini-Hochberg procedure) 校正 P 值 (P -adjust)。 P -adjust < 0.05 且 $|\log_2 \text{Fold change}| > 0$ 的基因被定义为 DEGs。随后利用京都基因和基因组百科全书 (KEGG, <http://www.genome.jp/kegg/pathway.html>) 数据库对上述结果进行富集注释, 分析 DEGs 列表显著富集的条目。

1.8 统计学方法

数据分析使用 SPSS 18.0 软件进行, 符合正态分布的计量资料采用 $\bar{x} \pm s$ 表示。采用两独立样本 t 检验进行组间比较。在进行 IL-17 信号通路中关键基因和下游炎症因子的组间差异分析时, 并未直接采用生信分析数据进行差异说明, 而是通过 RT-qPCR 实验进行验证后, 对 3 次实验的结果进行两独立样本 t 检验分析。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 小鼠脾脏指数检测

如表 2 所示, HST 组暴露 30 d 后, 与 PSC 组比较, 小鼠脾脏质量减少 ($P < 0.001$), 脾脏质量下降 ($P < 0.0001$), 脾脏指数降低 ($P < 0.05$)。结果表明, 高原低氧环境下小鼠脾脏组织质量减少。

2.2 小鼠脾脏组织病理学检测

HE 染色观察小鼠脾脏组织切片, 与 PSC 组相比, HST 组小鼠脾脏白髓体积减少, 生发中心范围扩大, 分界

表 2 小鼠脾脏指数检测

Table 2 Determination of the spleen index of the mice

Group	n	Spleen mass/g	Body mass/g	Spleen index/%
PSC	5	0.067±0.005	22.276±0.784	0.303±0.030
HST	5	0.044±0.005	18.300±0.759	0.242±0.031
t		7.061	8.143	3.160
P		<0.001	P<0.0001	P<0.05

PSC: plain spleen control; HST: hypoxic spleen test.

模糊, 并伴有静脉充血(图1)。提示高原低氧环境下小鼠脾脏组织发生炎症性病理学改变。

2.3 脾脏组织炎症因子IL-6、IL-12、IL-1β的mRNA和蛋白表达量检测

见图2。RT-qPCR检测结果发现, 高原低氧暴露下小鼠脾脏组织IL-6(P<0.05)、IL-12和IL-1β的mRNA表达量升

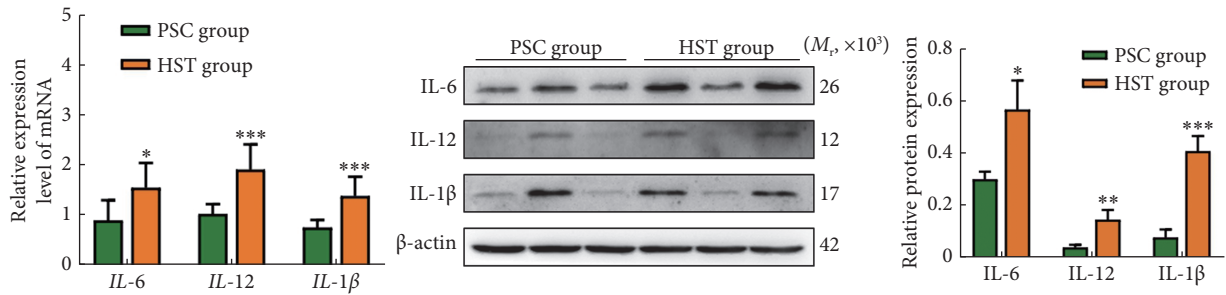


图 2 炎症因子IL-6、IL-12、IL-1β的mRNA和蛋白的相对表达量

Fig 2 Relative mRNA and protein expression levels of inflammatory cytokines IL-6, IL-12, and IL-1β

PSC: plain spleen control; HST: hypoxic spleen test. * P<0.05, ** P<0.01, *** P<0.001, vs. PSC group. n=5.

2.4 差异表达基因分析和KEGG富集分析

转录组测序共鉴定出4218个DEGs, 其中1950个基因表达上调, 2 268个基因表达下调(图3A)。进一步对DEGs进行KEGG通路富集分析, 4 218个DEGs共富集到

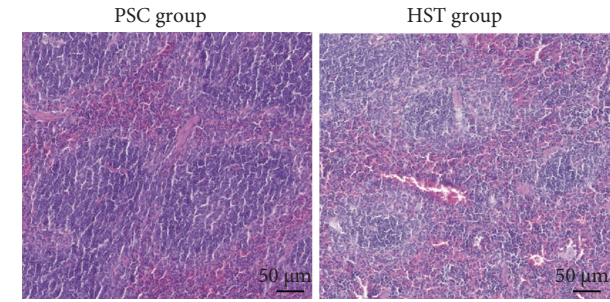


图 1 小鼠脾脏组织病理学改变 (HE染色)

Fig 1 Histopathological changes of the mouse spleen (HE staining)

PSC: plain spleen control; HST: hypoxic spleen test.

高(P<0.001)。Western blot发现IL-6、IL-12和IL-1β的蛋白表达均在高原低氧暴露下升高。结果表明, 高原低氧环境诱导小鼠脾脏组织炎症反应发生。

178个富集通路(P<0.05)。如图3B所示, 差异基因在T细胞受体信号通路、TNF信号通路和IL-17信号通路等多个与免疫、炎症相关通路中显著富集。说明高原低氧环境下小鼠脾脏组织发生了免疫失衡和炎症反应。

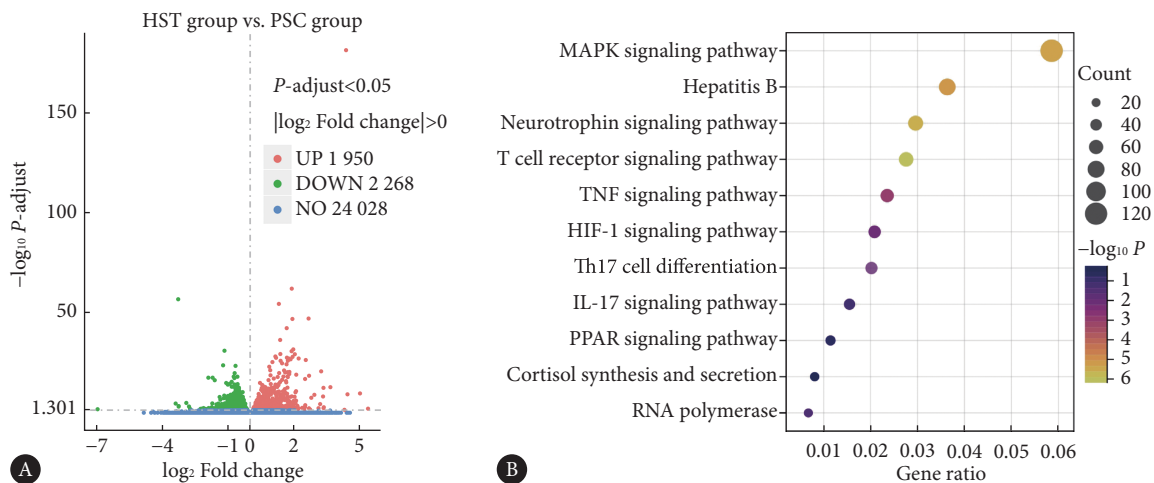


图 3 DEGs分析和KEGG通路富集分析

Fig 3 DEGs analysis and KEGG pathway enrichment analysis

PSC: plain spleen control; HST: hypoxic spleen test. A, Principal component analysis diagram; B, volcano map of DEGs.

2.5 IL-17信号通路验证

对通路进行分析,结果显示,许多差异基因与IL-17信号通路的特征靶基因高度相关。检测IL-17信号通路中DEGs的mRNA表达量,发现IL-17、IL-17R、ACT1、

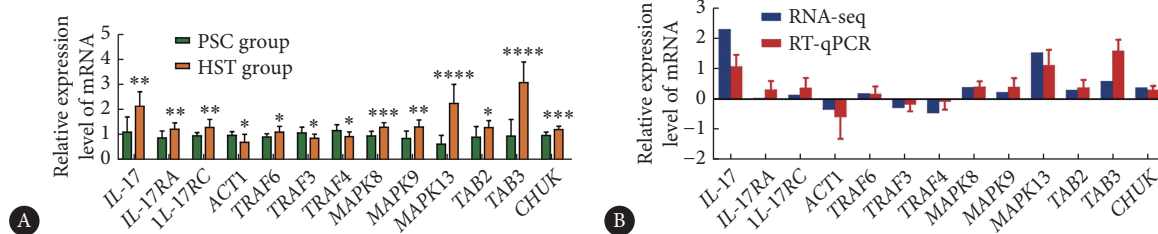


图 4 IL-17信号通路DEGs的mRNA表达量

Fig 4 mRNA expression of DEGs in IL-17 signaling pathway

PSC: plain spleen control; HST: hypoxic spleen test. A, Relative mRNA expression of DEGs in IL-17 signaling pathway; B, comparison of DEGs and RNA-Seq expression. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, vs. PSC group. $n = 5$.

2.6 IL-17信号通路下游炎症因子验证

对IL-17信号通路下游炎症因子CXCL5、TNF- α 、S100A8、S100A9、LCN2和MMP9的mRNA表达量进行检测。如图5所示,6个炎症因子的mRNA表达量在高原低氧环境下均上调。结果表明,高原低氧环境可促进IL-17信号通路释放下游炎症因子,从而介导小鼠脾脏组织炎症反应发生。

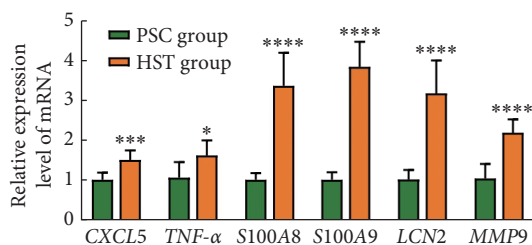


图 5 IL-17信号通路下游炎症因子的mRNA表达量

Fig 5 mRNA expression of inflammatory factors downstream of IL-17 signaling pathway

* $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$, vs. PSC group. $n = 5$. PSC: plain spleen control; HST: hypoxic spleen test.

3 讨论

高原低氧环境可诱导炎症反应的发生,并进一步激活免疫反应^[11]。研究发现,急性或慢性高原低氧环境均导致脾脏收缩,从而促进小鼠模型适应高原低氧环境^[12]。在本研究,通过构建高原低氧小鼠模型,发现相较于PSC组,HST组小鼠脾指数降低,脾脏组织白髓减少、生发中心扩大,边缘区模糊并伴有静脉充血,表明小鼠脾脏组织发生了炎症改变。研究发现,IL-6、IL-12和IL-1 β 在炎症反应的发生中起着至关重要的作用^[13]。其中,IL-6主要由单核/巨噬细胞、活化的淋巴细胞产生,并诱导炎症介

质作用的补体C3、C5aR等产生,从而介导局部炎症反应^[14]。WANG等^[15]发现在低氧环境下,促炎细胞因子IL-6和IL-12表达上调,可抑制T细胞免疫球蛋白和黏蛋白结构域(Tim)-3的表达,导致免疫失衡。IL-1 β 通过与STAT3诱导的细胞因子协同作用,诱导维甲酸受体相关孤儿受体 γ t(ROR- γ t)和IL-1R表达,维持Th17表型并增强Th17细胞的功能,从而在Th17参与的多种自身免疫性和慢性炎症性疾病中发挥作用^[16]。因此,本研究检测IL-6、IL-12、IL-1 β 的mRNA和蛋白表达量,发现相较于PSC组,HST组小鼠脾脏组织中IL-6、IL-12、IL-1 β 的mRNA和蛋白表达量均上调。以上结果提示,高原低氧环境下小鼠脾脏组织发生了炎症反应。

随后,本研究进行RNA-seq转录组学测序,以挖掘参与炎症反应调控的关键通路和基因。结果显示,高原低氧环境下小鼠脾脏组织中多个基因发生显著差异表达。通过KEGG通路富集分析,发现DEGs主要富集在T细胞受体信号通路、TNF信号通路和IL-17信号通路等多个与免疫、炎症相关通路。IL-17信号通路在宿主防御、组织修复和炎症性疾病发病机制中起着至关重要的作用。因此,重点关注IL-17信号通路在高原低氧环境诱导小鼠脾脏组织炎症反应发生的作用机制。IL-17信号通路主要由IL-17家族介导,IL-17A和IL-17F是主要的促炎症细胞因子,分别与IL-17RA和IL-17RC结合形成受体复合物,从而产生炎症靶基因的转录信号^[17]。IL-17受体复合物通过招募E3泛素连接酶ACT1,激活TNF受体相关因子(TRAF)^[18]。TRAF参与炎症反应的调节,主要由TRAF1-TRAF7组成。TRAF6是IL-17信号通路中的信号转导器,它可以与IL-17R和ACT1结合形成IL-17R-ACT1-TRAF6复合物,激活

NF- κ B和MAPK等信号通路发挥作用^[19]。TRAF3和TRAF4介导IL-17信号传导的负调节,抑制IL-17信号转导^[20]。本研究结果显示,在高原低氧环境下IL-17、IL-17RA、IL-17RC、TRAF6和MAPKs(MAPK8、MAPK9、MAPK13)的mRNA表达量均出现上调改变;ACT1、负调节基因TRAF3和TRAF4的mRNA表达量均出现下调改变。提示高原低氧环境激活小鼠脾脏组织IL-17信号通路。

IL-17信号通路激活可诱导趋化因子、促炎症细胞因子、抗菌蛋白和组织重构效应物释放,促进炎症反应的发生^[21]。相反敲除IL-17信号通路中的IL-17A,可通过抑制JAK通路而改善脾脏淀粉样变性,并缓解皮肤炎症反应^[22]。因此,进一步检测IL-17信号通路下游炎症因子变化。CXCL5是趋化因子家族成员,通过促进中性粒细胞浸润诱导组织炎症反应发生^[23]。本研究中CXCL5 mRNA表达上调,提示CXCL5可诱导炎症反应的发生。TNF- α 是一种促炎细胞因子,在炎症、细胞增殖和分化过程中发挥重要作用。本研究中,高原低氧环境下小鼠脾脏组织中TNF- α 表达上调,诱导组织发生炎症反应。在小鼠心肌细胞^[24]和大鼠脑组织^[25]的研究中也发现相似的结果。本研究结果显示S100A8、S100A9、LCN2的mRNA表达量在HST组显著升高。研究发现,LCN2是一种炎症介质,上调后通过募集炎症细胞、诱导炎症因子表达,促进炎症反应发生^[26]。S100A8/A9通过激活NF- κ B信号通路诱导小胶质细胞活化并促进促炎因子TNF- α 和MMP-9的产生^[27]。MMP-9主要由嗜中性粒细胞分泌,分泌过多会促进炎症发生^[28]。本研究发现,高原低氧环境下小鼠脾脏组织中MMP-9上调,诱导组织发生炎症反应。以上结果提示,高原低氧环境下IL-17信号通路下游炎症因子表达上调,导致小鼠脾脏组织炎症反应发生。

综上所述,高原低氧环境可以通过激活IL-17信号通路,促进下游炎症因子释放,诱导小鼠脾脏组织炎症反应发生。后续将进一步探讨高原低氧环境下抑制IL-17信号通路,对改善小鼠脾脏组织炎症反应发生的作用机制,为高原低氧环境对炎症反应发生机制提供新的实验依据。

* * *

作者贡献声明 永胜负责论文构思、经费获取和调查研究,郭玉静负责论文构思和审读与编辑写作,陈晓晨负责数据审编和调查研究,许玉珍负责正式分析,胡英负责经费获取和审读与编辑写作。所有作者已经同意将文章提交给本刊,且对将要发表版本进行最终定稿,并同意对工作的所有方面负责。

Author Contribution YONG Sheng is responsible for conceptualization, funding acquisition, and investigation. GUO Yujing is responsible for conceptualization and writing--review and editing. CHEN Xiaochen is responsible for data curation and investigation. XU Yuzhen is responsible

for formal analysis. HU Ying is responsible for funding acquisition and writing--review and editing. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

利益冲突 所有作者均声明不存在利益冲突

Declaration of Conflicting Interests All authors declare no competing interests.

参 考 文 献

- [1] MARTINELLO K A, MEEHAN C, AVDIC-BELLTHEUS A, *et al.* Acute LPS sensitization and continuous infusion exacerbates hypoxic brain injury in a piglet model of neonatal encephalopathy. *Sci Rep*, 2019, 9(1): 10184. doi: 10.1038/s41598-019-46488-y.
- [2] 任春贞, 骆亚莉, 刘永琦, 等. 黄芪百合颗粒对高原低氧模型小鼠肺自由基代谢及肺病理形态学改变的影响. *重庆医科大学学报*, 2017, 42(9): 1126-1130. doi: 10.13406/j.cnki.cyx.001010.
- [3] REN C Z, LUO Y L, LIU Y Q, *et al.* Effects of astragalus and lily particles on free radical metabolize and pathological changes of lung in mice under plateau hypoxia condition. *J Chongqing Med Univ*, 2017, 42(9): 1126-1130. doi: 10.13406/j.cnki.cyx.001010.
- [4] LEWIS S M, WILLIAMS A, EISENBARTH S C. Structure and function of the immune system in the spleen. *Sci Immunol*, 2019, 4(33): eaau6085. doi: 10.1126/sciimmunol.aau6085.
- [5] NORRIS P C, LIBREROS S, SERHAN C N. Resolution metabolomes activated by hypoxic environment. *Sci Adv*, 2019, 5(10): eaax4895. doi: 10.1126/sciadv.aax4895.
- [6] HANCKOVÁ M, MIHÁLIKOVÁ L, PASTOREKOVÁ S, *et al.* Hypoxia alters the immune response in mouse peritoneal macrophages infected with influenza A virus with truncated NS1 protein. *Cytokine*, 2023, 164: 156138. doi: 10.1016/j.cyto.2023.156138.
- [7] XU X, LI H, WEI Q, *et al.* Novel targets in a high-altitude pulmonary hypertension rat model based on RNA-seq and proteomics. *Front Med (Lausanne)*, 2021, 8: 742436. doi: 10.3389/fmed.2021.742436.
- [8] YANG M, WU S, CAI W, *et al.* Hypoxia-induced MIF induces dysregulation of lipid metabolism in Hep2 laryngocarcinoma through the IL-6/JAK-STAT pathway. *Lipids Health Dis*, 2022, 21(1): 82. doi: 10.1186/s12944-022-01693-z.
- [9] PANKOKE S, SCHWEITZER T, BIKKER R, *et al.* Obesity impacts hypoxia adaptation of the lung. *Am J Physiol Lung Cell Mol Physiol*, 2023, 325(3): L352-L359. doi: 10.1152/ajplung.00125.2023.
- [10] JOHNSON J, YANG Y J, BIAN Z L, *et al.* Systemic hypoxemia induces cardiomyocyte hypertrophy and right ventricular specific induction of proliferation. *Circ Res*, 2023, 132(6): 723-740. doi: 10.1161/Circresaha.122.321604.
- [11] 李慧倩, 胡英, 许玉珍, 等. 基于转录组测序技术研究高原低氧对小鼠脾脏组织的影响. *中国免疫学杂志*, 2023, 39(1): 11-17. doi: 10.3969/j.issn.1000-484X.2023.01.002.
- [12] LI H Q, HU Y, XU Y Z, *et al.* Study on effect of plateau hypoxia on mice spleen using transcriptome sequencing. *Chinese J Immunol*, 2023, 39(1):

- 11–17. doi: 10.3969/j.issn.1000-484X.2023.01.002.
- [11] MCGETTRICK A F, O'NEILL L A J. The role of HIF in immunity and inflammation. *Cell Metab*, 2020, 32(4): 524–536. doi: 10.1016/j.cmet.2020.08.002.
- [12] PURDY G M, JAMES M A, REES J L, *et al.* Spleen reactivity during incremental ascent to altitude. *J Appl Physiol* (1985), 2019, 126(1): 152–159. doi: 10.1152/jappphysiol.00753.2018.
- [13] STORJORD E, HENNO L T, MOLLNES T E, *et al.* Analysis of cytokines. *Tidsskr Nor Laegeforen*, 2020, 140(1). doi: 10.4045/tidsskr.18.0961.
- [14] KANG S, KISHIMOTO T. Interplay between interleukin-6 signaling and the vascular endothelium in cytokine storms. *Exp Mol Med*, 2021, 53(7): 1116–1123. doi: 10.1038/s12276-021-00649-0.
- [15] WANG W, XU Z, ZHANG J, *et al.* Tim-3 is a potential regulator that inhibits monocyte inflammation in response to intermittent hypoxia in children with obstructive sleep apnea syndrome. *Clin Immunol*, 2021, 222: 108641. doi: 10.1016/j.clim.2020.108641.
- [16] MANTOVANI A, DINARELLO C A, MOLGORA M, *et al.* Interleukin-1 and related cytokines in the regulation of inflammation and immunity. *Immunity*, 2019, 50(4): 778–795. doi: 10.1016/j.immuni.2019.03.012.
- [17] MEEHAN E V, WANG K. Interleukin-17 family cytokines in metabolic disorders and cancer. *Genes (Basel)*, 2022, 13(9): 1643. doi: 10.3390/genes13091643.
- [18] FENG Y, ZHENG C, ZHOU Z, *et al.* IL-17A neutralizing antibody attenuates eosinophilic meningitis caused by *angiostrongylus cantonensis* by involving IL-17RA/Traf6/NF- κ B signaling. *Exp Cell Res*, 2019, 384(1): 111554. doi: 10.1016/j.yexcr.2019.111554.
- [19] DU S, LI Z, XIE X, *et al.* IL-17 stimulates the expression of CCL2 in cardiac myocytes via Act1/TRAF6/p38MAPK-dependent AP-1 activation. *Scand J Immunol*, 2020, 91(1): e12840. doi: 10.1111/sji.12840.
- [20] SWAIDANI S, LIU C, ZHAO J, *et al.* TRAF regulation of IL-17 cytokine signaling. *Front Immunol*, 2019, 10: 1293. doi: 10.3389/fimmu.2019.01293.
- [21] CHUNG S H, YE X Q, IWAKURA Y. Interleukin-17 family members in health and disease. *Int Immunol*, 2021, 33(12): 723–729. doi: 10.1093/intimm/dxab075.
- [22] HIDA S, NAKANISHI T, MOMOSE F, *et al.* IL-17A is the critical cytokine for liver and spleen amyloidosis in inflammatory skin disease. *Int J Mol Sci*, 2022, 23(10): 5726. doi: 10.3390/ijms23105726.
- [23] ALVAREZ-COIRADAS E, MUNTEANU C R, DIAZ-SAEZ L, *et al.* Discovery of novel immunopharmacological ligands targeting the IL-17 inflammatory pathway. *Int Immunopharmacol*, 2020, 89(Pt A): 107026. doi: 10.1016/j.intimp.2020.107026.
- [24] WEN X, YIN Y, LI X, *et al.* Effect of miR-26a-5p targeting ADAM17 gene on apoptosis, inflammatory factors and oxidative stress response of myocardial cells in hypoxic model. *J Bioenerg Biomembr*, 2020, 52(2): 83–92. doi: 10.1007/s10863-020-09829-5.
- [25] WU Y, WEI H, LI P, *et al.* Quercetin administration following hypoxia-induced neonatal brain damage attenuates later-life seizure susceptibility and anxiety-related behavior: modulating inflammatory response. *Front Pediatr*, 2022, 10: 791815. doi: 10.3389/fped.2022.791815.
- [26] GUARDADO S, OJEDA-JUAREZ D, KAUL M, *et al.* Comprehensive review of lipocalin 2-mediated effects in lung inflammation. *Am J Physiol Lung Cell Mol Physiol*, 2021, 321(4): L726–L733. doi: 10.1152/ajplung.00080.2021.
- [27] WU M, XU L, WANG Y, *et al.* S100A8/A9 induces microglia activation and promotes the apoptosis of oligodendrocyte precursor cells by activating the NF- κ B signaling pathway. *Brain Res Bull*, 2018, 143(12): 234–245. doi: 10.1016/j.brainresbull.2018.09.014.
- [28] DONG Y, ZHAO H, MAN J, *et al.* MMP-9-mediated regulation of hypoxia-reperfusion injury-related neutrophil inflammation in an *in vitro* proximal tubular cell model. *Ren Fail*, 2021, 43(1): 900–910. doi: 10.1080/0886022X.2021.1930558.

(2023 – 05 – 20收稿, 2024 – 01 – 02修回)

编辑 吕熙



开放获取

Open Access

© 2024 《四川大学学报(医学版)》编辑部 版权所有
Editorial Office of *Journal of Sichuan University (Medical Science)*