危重症学组

Enhanced Biosynthesis of Fatty Acids Contributes to Ciprofloxacin Resistance in Pseudomonas aeruginosa

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Abstract

Antibiotic-resistant Pseudomonas aeruginosa is insensitive to antibiotics and difficult to deal with. An understanding for the resistance mechanisms is required for control of the pathogen. Here, GC-MS based metabolomics was performed to identify differential metabolomes in ciprofloxacin-resistant P. aeruginosa strains that originated from ATCC 27853 and had MICs that were 16-, 64- and 128-fold (PA-R16CIP, PA-R64CIP and PA-R128CIP, respectively) higher than the original value, compared to ciprofloxacin-sensitive P. aeruginosa (PA-S). Upregulation of fatty acid biosynthesis forms a characteristic feature of the ciprofloxacin-resistant metabolomes and fatty acid metabolome, which was supported by elevated gene expression and enzymatic activity in the metabolic pathway. The fatty acid synthase inhibitor triclosan potentiates ciprofloxacin to kill PA-R128CIP and clinically multidrug-resistant P. aeruginosa strains. The potentiated killing was companied with reduced gene expression and enzymatic activity and the returned abundance of fatty acids in the metabolic pathway. Consistently, membrane permeability was reduced in the PA-R and clinically multidrug-resistant P. aeruginosa strains, which was reverted by triclosan. Triclosan also stimulated uptake of ciprofloxacin. These findings highlight the importance for the elevated biosynthesis of fatty acids in ciprofloxacin resistance of P. aeruginosa and provide a target pathway for combating ciprofloxacin-resistance P. aeruginosa.

Keywords: Pseudomonas aeruginosa; antibiotic resistance; biosynthesis of fatty acids; ciprofloxacin; membrane permeability; metabolomics

肌醇激活自噬抑制 HIF-1α/SLUG 信号通路缓解急性呼吸窘迫综合征肺纤维化

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【摘 要】

目的：探讨肌醇缓解急性呼吸窘迫综合征（ARDS）肺纤维化的作用和机制。

方法：通过脂多糖（LPS）在体内建立 ARDS 小鼠模型和在体外建立肺泡上皮细胞炎症模型，同时添加肌醇进行干预。用病理学法检测肺组织的病理变化，用免疫荧光和免疫印迹法检测自噬基因以及纤维化相关基因的表达。通过 mRNA-seq 分析肌醇干预后 ARDS 的调节信号通路的变化，并通过报告基因以及相关实验进行验证。

结果：体内组织标本的 HE 和 masson 染色表明，肌醇可以缓解 ARDS 肺纤维化，肌醇促进细胞上皮细胞间充质转化（EMT）的关键基因 E-Cadherin 的表达，并抑制 N-Cadherin 的表达。免疫荧光和 western blot 实验证实，肌醇可以激活自噬的产生，同时受肌醇调控。mRNA-seq 分析显示，HIF-1α/SLUG 信号通路参与细胞的 EMT 进展，并被肌醇诱导的自噬抑制。

结论：肌醇可以激活自噬用以抑制 ARDS 中 HIF-1α/SLUG 信号通路诱导的 EMT 进展，进而缓解肺纤维化。

关键词：肌醇；急性呼吸窘迫症；HIF-1α/SLUG；EMT

Inositol activates autophagy to inhibit HIF-1α/SLUG signaling pathway and alleviate fibrosis in acute respiratory distress syndrome

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Abstract

Objective：The purpose of this study was to investigate the role and mechanism of inositol in the development of acute respiratory distress syndrome (ARDS), and to explore clinical drugs for ARDS.

Methods：Lipopolysaccharide (LPS) established ARDS mouse model in vivo and alveolar epithelial cell inflammation model in vitro, and added inositol for intervention. The pathological changes of lung tissue were detected by pathology, and the expressions of autophagy genes and fibrosis-related genes were detected by immunofluorescence and western blotting. Changes in ARDS-regulated signaling pathways affected by inositol were analyzed by mRNA-seq and validated by reporter genes and related experiments.

Results：HE and masson staining of tissue specimens in vivo showed that inositol could alleviate ARDS-induced pulmonary fibrosis, and the expression of E-Cadherin, a key gene of cell epithelial-mesenchymal transition (EMT), was promoted by inositol, and N- Cadherin expression is inhibited by inositol. Immunofluorescence and western blot experiments confirmed that inositol can activate the production of autophagy and is regulated by inositol. mRNA-seq analysis showed that the HIF-1α/SLUG signaling pathway was involved in EMT progression in cells and was inhibited by inositol-induced autophagy.

Conclusions Inositol can activate autophagy to inhibit the progression of EMT induced by HIF-1α/SLUG signaling pathway in ARDS, thereby alleviating pulmonary fibrosis.

Key words： inositol; acute respiratory distress syndrome; HIF-1α/SLUG; EMT Fund program：Science and Technology Projects in Guangzhou（No.202102080226） Science and Education Projects of Guangzhou Health Commission(No.20201A010019)

Microglial exosomal miR-466i-5p induces brain injury via promoting hippocampal neuron apoptosis in heatstroke

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Abstract

Background： Brain injury in severe heatstroke (HS) is a common and important clinical issue, which is an important cause of prognosis in patients, and neuronal apoptosis is an important aspect. However, the pathogenesis of HS-induced neuronal apoptosis is not fully elucidated. As critical immune cells in the brain, microglia can regulate the function of target cells by secreting exosomes, which contains diverse functional microRNA (miRNA) cargos. However, whether microglial miRNA are involved in HS-induced neuron apoptosis has not been elucidated. This work aimed to clarify the differently expressions of microglial exosomal miRNAs under HS and to explore their role in mediating HS-induced neuron apoptosis.

Methods： HS mice model is established in a pre-warmed artificial climate chamber with a temperature of 35.5±0.5˚C and relative humidity of 60±5%. The rectal core temperature (Tc) is monitored. Heat stress (HS) is halted at Tc of more than 41˚C. HS HT22 is maintained at 42℃ for 2h, while the control is maintained at 37℃. The microglial exosomes are isolated by standard differential ultracentrifugation and characterized. We analyzed expressed Mir-466i-5p differences of the control and HS exosomes by high-throughput sequencing and further conducted gene ontology (GO) pathway analysis. Recipient neurons are treated with the control or the HS exosomes, whereas in vivo, the exosomes were injected into mice. The internalization of HS microglial exosomes by neurons was tracked. The effect and mechanism of HS exosomal miR-466i-5p on the induction of neuron apoptosis are demonstrated in vitro.

Results： HS-induced an increase in neurons apoptosis. Microglial exosomes are identified and taken up by neurons in vitro and vivo. Microglial exosomes induced HT22 apoptosis. HS significantly changed the miRNA profiles of microglial exosomes based on high-throughput sequencing. We selected miR-466i-5p as a target. Microglial exosomes with upregulated miR-466i-5p induced neurons apoptosis in vitro experiments. The effects are exerted by miR-466i-5p target Bcl-2, activating caspase-3 to induce neurons apoptosis.

Conclusions： This work is the first preliminary study to demonstrate the effect of microglial exosomal miR-466i-5p on neurons apoptosis and reveals potentially Bcl-2/caspase-3 in heatstroke. These results provide a basis for future research on the protection of brain injury in heatstroke and the identification of new targets for clinical treatment.

Key words: heatstroke, microglia, exosome, miR-466i-5p, neuron apoptosis

单中心PICU496例重症伤害患儿回顾分析

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【摘 要】

目的：分析儿童危重症伤害的疾病分布、发生时间、地点等特征，为预防儿童伤害提供参考，以制定有效预防措施。

方法：回顾分析深圳市儿童医院 PICU 在 2016 年 01 月 01 日至 2021 年 12 月 31 日期间收治的危重症儿童伤害病例的临床资料，对伤害组成、性别、年龄、发生地点及时间、预后等流行病学进行分析。

结果：496 例危重症伤害患儿中，故意伤害 35 例（7.0%），意外伤害 461 例（93.0%）。故意伤害中性别比例：男性 19 例，女性 16 例，构成比为 1.3:1；伤害类型主要为自杀 23 例（65.7%），女孩以服用药物为主，男孩以坠落伤为主，故意伤害以学龄期儿童以后 23 例（65.7%）；故意伤害有逐年增加趋势，新冠疫情后增加明显。意外伤害前三位依次为坠落/摔伤 151 例（31.5%）、交通伤 129 例（29.5%）、异物 70 例（15.5%）；意外伤害中男性占 284 例，女性占 177 例，构成比为 1.6:1；意外伤害学龄期前儿童（330 例，71.6%），意外伤害发生季节无明显特征，但淹溺与烫伤易发生夏秋季节；16-21 时最易发生意外伤害 219 例（47.5%），伤害发生地点家庭 276 例（60.0%），公路 109 例（23.6%）；疾病负担方面意外伤害多器官功能衰竭 39 例（8.5%），多发伤 109 例（23.6%），平均住院天数 25.4 天，平均住院费用 54747.03 元，致残/致畸 145 例（31.5%），死亡 51 例（11.0%）。

结论：儿童危重症伤害以意外伤害为主，坠落伤及交通伤、异物更容易发生，男性患儿风险大，意外伤害以学龄期以前儿童为主，故意伤害以学龄期以后儿童为主，伤害主要在 16-21 时，地点多为家庭。伤害对患儿危害大，疾病负担重，致死、致残率高。应针对上述特点，建立具有针对性的防治体制，以提高防治效率。

先天性心脏病术后血流感染病原菌分布及耐药性分析

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【摘　要】

目的：了解先天性心脏病（Congenital Heart Disease，CHD）术后血流感染(Bloodstream Infection，BSI)的病原菌分布及其耐药性，以指导临床合理用药。

方法：回顾性分析 2012 年 1 月至 2021 年 12 月广东省人民医院 CHD 术后 BSI 患儿微生物学和药物敏感试验资料。

结果：76 例 BSI 患儿共检出 77 株病原菌，其中革兰阳性（G+）菌 44 株（57.14%），革兰阴性（G-）菌 25 株（32.47%），真菌 8 株（10.39%）；男女比例为 1.81:1，男 49 例(64.47%)，女 27 例(35.53%)；1-6 月龄 59 例(77.63%)，7-12 月龄 9 例(11.84%)，>1 岁 8 例（10.53%），年龄中位数为 3.0（1.0，6.0）个月；简单型 CHD 42 例（55.26%），复杂型 CHD34 例（44.74%）；术后<7 天发生 BSI 37 例（48.68%），7-14 天 22 例（28.95%），>14 天 17 例（22.37%）；复杂型 CHD 术后发生真菌 BSI 的比例显著高于简单型 CHD（P＜0.05）；引起 BSI 主要病原菌分别为表皮葡萄球菌（19 株，24.68%）、金黄色葡萄球菌（11 株，14.29%）、肺炎克雷伯杆菌（11 株，14.29%）、鲍曼氏不动杆菌（4 株，5.19%）、溶血葡萄球菌（4 株，5.19%）、粪肠球菌（4 株，5.19%）、白色念珠菌（3 株，3.90%）和产气肠杆菌（3 株，3.90%）；G+菌 BSI 中以表皮葡萄球菌为主，且对万古霉素的耐药率为 0. 00%，G-菌 BSI 中以肺炎克雷伯杆菌为主，且对碳青霉烯类最为敏感，真菌 BSI 则以白色念珠菌为主，且对氟康唑、两性霉素 B、5-氟胞嘧啶、伏立康唑、伊曲康唑 100%敏感；存活出院 74 例（97.37%），院内死亡 2 例（2.63%）。

结论:CHD 术后 BSI 多见于 1-6 月龄男性婴儿，且多见于术后头一周；BSI 主要病原菌为表皮葡萄球菌、肺炎克雷伯杆菌和白色念珠菌；糖肽类、碳青霉烯类抗生素和唑类抗真菌药物分别为 G+菌、G-菌和真菌 BSI 治疗的首选。

小檗碱对脂多糖气道给药诱导的大鼠急性肺损伤的作用及机制

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【摘 要】

目的:观察小檗碱对脂多糖气道给药诱导的大鼠急性肺损伤的作用并探讨其机制。

方法:1、气道内滴注LPS建立大鼠急性肺损伤模型；2. 30只4~6周龄雄性SD大鼠随机分为3组（每组10只），对照组、模型组和干预组。干预组分别于造模前48h和24h、造模后2h腹腔注射BBR 10mL/kg（1mg/mL）。3组大鼠均于造模后24h处死，处死时收集血清和肺组织。3. 检测大鼠血和肺组织炎症情况、检测肺组织氧化应激、细胞凋亡及自噬情况。

结果:1.气道内滴注 LPS 成功建立大鼠急性肺损伤模型

造模 24h 后处死，显微镜下观察肺组织病理：可见肺泡充血、肺泡间隔增厚、肺透明膜形成，并伴有大量炎症细胞浸润。判断气道内滴注 LPS 建立大鼠急性肺损伤模型成功。

2.各组大鼠血和肺组织炎症情况

模型组相比，肺组织 HE 染色可见干预组肺水肿减轻、肺泡的炎症细胞减少。干预组血清和肺组织匀浆 TNF-α、IL-1β 和 IL-6 水平较模型组降低（$P<0.05$）。同时干预组肺组织 TNF-α、IL-1β、IL-6 和 RAGE mRNA 水平较模型组降低（$P<0.05$）。

3.各组大鼠肺组织氧化应激情况

与模型组相比，干预组肺组织 SOD 水平上升，而 MDA 有所下降，差异有统计学意义（$P<0.05$）。

4．各组大鼠肺组织细胞凋亡情况

与模型组相比，干预组肺组织 Bcl-2 mRNA 水平增高、Bax mRNA 水平下降，Bcl-2/Bax 水平升高（$P<0.05$）；干预组肺组织 C-Caspase 3 表达下降，Bcl-2 / Bax 增加（$P<0.05$）；同时干预组肺组织 Tunel 荧光强度减弱，凋亡指数下降（$P<0.01$）。

5．各组大鼠肺组织自噬情况：干预组 LC3II、LC3II/p62 和 Beclin-1 mRNA 水平较模型组均有降低（$P<0.05$）。

模型组肺组织 Lamp2 和 LC3II 荧光强度较对照组增加（$P<0.0001$）。与模型组相比，干预组肺组织 Lamp2 和 LC3II 荧光强度减弱（$P<0.05$）。

结论： 1、气道滴注脂多糖成功构建了急性肺损伤大鼠模型。

2、小檗碱可通过降低炎症反应、减轻氧化应激、减少细胞凋亡而减轻模型大鼠的急性肺损伤。

干预组大鼠肺组织自噬相关基因 LC3II、LC3II/p62、Beclin-1 mRNA 水平下降，LC3II 和 Lamp2 蛋白表达减少，提示小檗碱可能通过抑制自噬发挥对肺损伤的保护作用，但是具体机制有待进一步探究。