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微小 RNA 调控脓毒症患者外周血免疫因子表达及其机制研究 *

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摘要 目的：探讨脓毒血症患者血清外周白细胞中的微小 RNA(miRNA)表达水平的变化及其在脓毒症患者中的表达意义以及免疫调控的关系。**方法：**采用流式细胞仪检测外周血 $CD4^+CD25^+$Treg 细胞表达，采用实时定量 PCR(RT-PCR)方法检测 110 例脓毒症患者以及 100 例正常对照外周血白细胞中 miRNA 以及 Foxp3 mRNA 表达量，酶联免疫吸附法测定 TNF-α 和 IL-10 浓度，序贯器官衰竭估计(SOFA)评分系统评价脓毒症患者的严重程度。对 miRNA 与白细胞总数、TNF-α、IL-10 和 SOFA 评分之间的相关性进行分析。**结果：**实验组 miRNA 表达水平较对照组显著降低(P<0.01)，WBC、IL-10 水平显著升高(P<0.01)。实验组 miRNA 表达水平以及 SOFA 评分、血清 TNF-α 和 IL-10 之间呈负相关关系(r 值分别为 -0.512、-0.623、-0.432，P<0.05)；与 WBC 无显著相关性(r=0.215，P>0.05)。脓毒症患者外周血 Treg 表达率和 Foxp3mRNA 均显著高于对照组(P<0.01)；随病情严重而升高，轻、中、重度实验组间两两比较差异均有统计学意义(P<0.01)。死亡均在重度脓毒症患者中，死亡组 Treg、Foxp3 mRNA 及 IL-10 均显著高于存活组(P<0.01)；miRNA 低于存活组(P<0.01)。**结论：**脓毒症患者外周血的 miRNA 表达量显著降低，表达水平在一定程度上可以反应机体的炎症反应情况，同时还可以判断疾病的严重度，且 miRNA 参与对 Treg 细胞增殖的调节，在脓毒症免疫失衡机制中发挥一定的作用。

关键词：微小 RNA；肿瘤坏死因子 -α；白细胞介素 -10；免疫调节

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Clinical Significance of Small RNA Expression in Peripheral Blood of Patients with Sepsis and Its Influence on Immune Regulation Mechanism*

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ABSTRACT Objective: To observe the clinical significance of Small RNA expression in peripheral blood of patients with sepsis and its influence on immune regulation mechanism. **Methods:** 110 cases of sepsis patients were selected as study group and another 100 healthy cases were as controls. Flow cytometry instrument was used to detect peripheral blood $CD4^+CD25^+$ Treg cells expression, real-time quantitative PCR (RT-PCR) was used to detect miRNA and Foxp3 mRNA expression in the peripheral blood, enzyme-linked immunosorbent was to detect concentration of TNF-α and IL-10, and sequential organ failure estimation (SOFA) score system was used to evaluate the severity of the patients with sepsis. The correlation of miRNA with total number of white blood cells, the TNF-α, IL-10 and SOFA score were analyzed. **Results:** The expression level of miRNA in study group was lower than that of the control group (P<0.01), and the level of WBC and IL-10 was significantly increased (P<0.01). miRNA expression and SOFA score, serum TNF-α in patients with sepsis had a negative correlation with IL-10 (r = -0.512 -0.623, -0.432, P<0.05); there was no significant correlation with the WBC (r=0.215, P>0.05). Treg expression rate and Foxp3mRNA in peripheral blood of patients with sepsis were significantly higher than that of healthy controls (P<0.01) and increased along with the severity of disease, and there was statistical significance in comparison between slight, moderate and severe sepsis (P<0.01). Death patients appeared in the patients with severe sepsis, Treg, Foxp3 mRNA and IL-10 levels in death patients were significantly higher than survival patients (P<0.01); miRNA in death patients was lower than that of survival patients (P<0.01). **Conclusions:** MiRNA expression is decreased significantly in peripheral blood of patients with sepsis, miRNA level can not only reflect the state of inflammatory response of the body, and to a certain extent, can be used as index to judge the severity of disease and prognosis, miRNA is also involved in regulation of Treg cells proliferation, and play a role in sepsis immune mechanism.

Key words: MicroRNA; Tumor necrosis factor; Interleukin 10; Immune regulation

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前言

脓毒血症是由于各类的致病微生物在体内存积导致机体出现炎性状态的一种全身炎症性反应综合征(SIRS),若没有进行及时处理则会出现多器官功能衰竭,危及生命,影响预后[1,2]。微小 RNA(miRNA)是近些年来科学家最新发现的一种起到转录后调节作用的基因,与靶基因 mRBA 结合可调节其他基因及蛋白的 mRNA 表达,并且广泛参与体内的炎症、器官移植、肿瘤等疾病的发生过程[3]。研究发现,miRNA 可能从基因水平参与了脓毒血症的发病,可以用于脓毒血症严重程度的判断[4]。我们对 miRNA 在脓毒症患者外周血中表达的临床意义及免疫调控机制作进行了相关研究,现汇报如下。

1 材料与方法

1.1 研究对象

选取 2010 年 4 月至 2014 年 11 月我院重症监护室(ICU)收治的脓毒症患者 110 例为实验组,其诊断均依据美国胸科医师学会 / 危重病医学会(ACCP/SCCM)在 1992 年所提出的关于脓毒症的相关诊断标准,根据急性生理学以及慢性健康状况评分系统(APACHE II),其中轻度 35 例,中度 42 例,重度 33 例;男 78 例,女 32 例;年龄 21～77 岁,平均(53.4± 15.6)岁。同时选取门诊健康体检者 100 例为对照组,其中男 32 例,女 18 例;年龄 24-71 岁,平均(53.4± 4.2)岁。两组性别、年龄等基本资料差异无统计学意义(P>0.05),具有可比性。

1.2 研究方法

患者在确诊后 2 h 内抽取静脉血 6 mL,应用肝素抗凝。2 mL 用于流式细胞仪检测分析,2 mL 用于检测 miRNA 和 Foxp3 mRNA 表达水平,2 mL 用于细胞因子检测。应用流式细胞仪方法检测 $CD4^+CD25^+$Treg 细胞,在荧光定量 PCR 仪上进行 RT-PCR 方法检测 miRNA 和 Foxp3 mRNA 表达水平:采用 ELISA 检测血浆中 TNF-α 及 IL-10 含量,根据说明书进行操作。SOFA 评分特点在于:寻找一个客观而简单的方法并能以连续的形式描述单个器官的功能障碍或衰竭,同时能评价从轻微的功能障碍到重度衰竭的程度,能在临床研究中反复计量单个或全体器官功能障碍的发生发展,由此确定描述器官功能障碍或衰竭的特征。故本实验采用国际通用 SOFA 评分,应用 SOFA 评分表,对患者以连续的形式进行单个器官功能进行评价[5]。

1.3 统计学处理

应用 SPSS19.0 统计软件进行分析处理,计量资料以($\bar{x}\pm s$)表示,采用 t 检验,计数资料用相对数表示,采用 X^2 检验。检验标准为 0.05,P<0.05 时差异有统计学意义。

2 结果

2.1 脓毒症患者病情分析

110 例脓毒症患者中出现 32 例肺部感染,17 例腹膜炎例,3 例胆道感染,11 例细菌性心内膜炎。病死率为 11.82 %(13/110);SOFA 评分 3-20 分,平均(9.3± 3.2)分。

2.2 外周血 miRNA、WBC、血清 TNF-α、IL-10 水平

实验组 miRNA 表达水平较正常对照组显著降低,差异具有统计学意义(P<0.01);实验组 WBC、TNF-α 和 IL-10 水平较正常对照组显著升高,差异具有统计学意义(P<0.01)。见表 1。

表 1 两组患者 miRNA,WBC,TNF-α 及 IL-10 水平变化情况比较

Table 1 Comparison of changes of miRNA, WBC, TNF-α and IL-10 between two groups

Group	Case	miRNA	TNF-α (pg/mL)	IL-10 (pg/mL)	WBC (× 10^9/L)
Study group	55	1.05± 0.29	154.13± 6.39	127.94± 5.28	11.18± 1.23
Control group	55	1.85± 0.12	45.28± 4.98	37.38± 4.28	6.78± 1.82
t		29.34	3.02	33.13	2.88
P		<0.05	<0.05	<0.05	<0.05

2.3 脓毒症患者 WBC、TNF-α、IL-10 及 SOFA 评分的关系

脓毒症患者 miRNA 表达量与血清 TNF-α、IL-10 和 SOFA 评分之间呈负相关关系(r 值分别为 -0.623、-0.432、-0.512,P<0.05);与 WBC 无显著相关性(P>0.05)。

表 2 miRNA 与 TNF-α 和 IL-10 及 WBC 线性关系表

Table 2 Linear relation of MiRNA and TNF- alpha and IL-10 and WBC

	TNF-α (pg/mL)	IL-10 (pg/mL)	WBC (× 10^9/L)	SOFA
r	-0.623	-0.432	0.215	-0.512
P	P<0.05	P<0.05	P>0.05	P<0.05

2.4 外周血 Treg 表达率和 Foxp3mRNA 水平

脓毒症患者外周血 Treg 表达率和 Foxp3 mRNA 均显著高于健康对照组 (P<0.01);且随疾病严重度的升高而逐渐升高,轻、中、重度实验组间两两比较差异均有统计学意义(P<0.01)。

2.5 死亡患者及存活患者外周血 Treg,miRNA,Foxp3 mRNA 以及 IL-10 表达

死亡患者均出现在重度脓毒症患者中,死亡组 Treg、Foxp3 mRNA 及 IL-10 均显著高于存活组,差异具有统计学意义(P<0.01);与死亡组比较,生存组 miRNA 值较高,差异具有统计学意义(P<0.01)。见表 3。

3 讨论

表 3 不同病情严重度脓毒症患者的外周血 Treg 表达率以及 Foxp3 表达水平

Table 3 Treg expression rate and Foxp3 expression level of peripheral blood in different illness severity of sepsis patients

Disease stages	Case	Treg	Foxp3 mRNA
Slight	33	2.62± 0.78[c]	0.14± 0.03[c]
Moderate	42	2.88± 0.89[a,c]	0.19± 0.01[a,c]
Severe	35	3.01± 1.21[a,b,c]	0.22± 0.02[a,b,c]
Control group	100	1.28± 0.14	0.04± 0.01

Note: compared with slight group, aP<0.05; compared with moderate group, bP<0.05; compared with control group cP<0.05

表 4 死亡患者以及存活患者的外周血 Treg 表达以及 miRNA、Foxp3 mRNA 表达以及 IL-10 表达

Table 4 Expressions of Treg, miRNA, Foxp3 mRNA and IL-10 of peripheral blood in death and survival patients

Group	Case	Treg (%)	miRNA	Foxp3 mRNA	IL-10
Survival group	88	2.88± 1.04	0.95± 0.17	0.18± 0.03	51.77± 5.74
Death group	22	3.44± 0.54	0.42± 0.05	0.23± 0.06	65.29± 13.86
t		3.44	2.71	3.97	3.84
P		<0.05	<0.05	<0.05	<0.05

脓毒症是一类由于病原微生物感染所导致的的 SIRS，其发生机制是机体促炎以及抗炎机制的失衡所导致的。既往研究指出，miRNA 在转录后对于机体的炎症信号通路水平可以起到调控作用而调控失衡的炎症反应[6-8]。

IL-10 是一种主要的抗炎因子，高水平的 IL-10 及肿瘤坏死因子 -α(TNF-α)是脓毒血症患者死亡的独立危险因素[9, 10]。研究表明，脓毒症患者的 IL-10 水平可以反映免疫及疾病危重程度[11]。脓毒症患者早期体内升高的 miRNA 对感染有益，从而促进机体促炎细胞因子、特异性抗体以及效应性 T 细胞的形成，但过度激活将导致促炎因子的大量释放，引起免疫细胞的过度活化，同时 miRNA 可以作用于 Treg 细胞，参与免疫抑制[12-14]。随疾病进展，miRNA 调节 Treg 的增殖能力加强，抗炎反应呈现失控性增强，抗炎反应占据优势，发生免疫麻痹，将导致休克甚至死亡[15-17]。本研究结果显示，脓毒症患者外周血 Treg、Foxp3 表达水平明显高于正常对照组（P<0.05）。结果说明，脓毒症发生后机体发生了强烈的炎性反应，但促炎反应过强则会使其破坏性增强。提示我们，Treg 和 IL-10 的代偿性升高可以起到对抗炎症反应的效果，对机体起到一定的保护作用。此外，死亡组 miRNA 水平明显低于存活组（P<0.05）。结果说明，miRNA 表达水平与患者预后有一定关系，表达水平越低表示预后越差。进一步分析发现，Treg、Foxp3 及 IL-10 表达水平随病情加重而升高（P<0.05）。结果表明，miRNA 与 Treg 过表达影响着脓毒症的发生及发展。

SOFA 可以更加方便地收集数据，动态监测疾病的进展，SOFA 评分有助于描述患者的器官功能不全甚至衰竭的整个过程。研究指出最高 SOFA 评分与病情呈正相关，其对于病情的评价有着很好的预后价值 [18-20]。本研究发现，脓毒症患者 miRNA 的表达量与 SOFA 评分之间呈负相关关系（P<0.05）。结果说明，miRNA 表达水平与疾病严重程度有关，miRNA 水平越低，患者病情越重。我们还发现，患者 miRNA 表达水平与 TNF-α 和 IL-10 呈负相关，且与 WBC 无关（P>0.05）。说明其表达可以反应机体的炎症水平，与 WBC 无关则可以避免由于抗生素的使用 WBC 降低而对疾病诊断产生的影响。提示我们，脓毒症患者外周血中 miRNA 表达量显著降低，并且与外周血白细胞总数无关。

综上所述，miRNA 水平不仅可以反映机体炎症反应状态，而且在一定程度上可以评判疾病的预后及严重程度。同时，miRNA 参与调节 Treg 细胞增殖，在脓毒症免疫失衡机制中发挥一定的作用。miRNA 可能成为脓毒症治疗的一个新靶点。

参 考 文 献（References）

[1] Saadia A, Asia M, Freed A, et al. Mi-DISCOVERER: A Bioinformatics Tool Detection of mi-RNA in Human Genome [J]. Bioinformation, 2011, 5(6): 562-563

[2] Kanwal N, John P, Bhatti A. MicroRNA-155 as a therapeutic target for inflammatory diseases[J]. Rheumatol Int, 2013, 33(3): 557-560

[3] Su XQ, Xing JD, Wang ZZ, et al. microRNAs and ceRNAs: RNA networks in pathogenesis of cancer [J]. China cancer research, 2013, 25(2): 235-239

[4] Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS [J]. Crit Care Med, 1996, 24(7): 1125-1128

[5] Wang H, Zhang P, Chen W, et a1. Serum microRNA signatures identified By Solexa sequencing predict sepsis patients'mortality：a prospective Observational study[J]. PLoS One, 2012, 7(6): e38885

[6] Ferrajoli A, Shanafelt TD, Ivan C, et a1. Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and Patients with B chronic lymphocytic leukemia[J]. Blood, 2013, 16(9): 122-123

[7] Sun Kai, Su Gui-yuan, Deng Hai-jun, et al. Relationship between miRNA-338-3p expression and progression and prognosis of human colorectal carcinoma[J]. Chinese Journal of Medicine, 2014, 127(10): 1884-1890

[8] Zhang ZZ, Liu X, Wang DQ, et al. Hepatitis B virus and hepatocellular carcinoma at the miRNA level[J]. World Journal of Gastroenterology, 2011, 6(1): 76-77

[9] Florian M, Udo H. Mechanisms of Lin28-Mediated miRNA and

mRNA Regulation-A Structural and Functional Perspective [J]. International Journal of Molecular Sciences, 2013, 14(8): 754-756

[10] Mian G, Liu W, Meng Q, et al. Identification of microRNAs in six solanaceous plants and their potential link with phosphate and mycorrhizal signalings[J]. Plant Acta(English edition), 2014, 14(12): 1164-1178

[11] Li WY, Chen XM, Xiong W, et al. Detection of Microvesicle miRNA Expression in ALL Subtypes and Analysis of Their Functional Roles [J]. Acta of Huazhong University of Science and Technology(English edition), 2014, 34(5): 640-645.

[12] Pedro MC, Maria CP. MicroRNAs as Molecular Targets for Cancer Therapy: On the Modulation of MicroRNA Expression [J]. Pharmaceuticals, 2013, 6(10): 134-135

[13] Chen JP, Qin ZZ, Pan SD, et al. Genetic variants in RAN, DICER and HIWI of microRNA biogenesis genes and risk of cervical carcinoma in a Chinese population[J]. China cancer research, 2013, 25(5): 565-571

[14] Li Pei-pei, Wang Xin. Role of signaling pathways and miRNAs in chronic lymphocytic leukemia[J].Chinese Journal of Medicine, 2013, 126(21): 4175-4182

[15] Li Wen-bin, Chen Hui-yuan, Zhang Wei, et al. Relationship between magnetic resonance imaging features and miRNA gene expression in patients with glioblastoma multiforme [J]. Chinese Journal of Medicine, 2013, 126(15): 2881-2885

[16] Wei J, Gao W, Zhu CJ, et al. Identification of plasma microRNA-21 as a biomarker for early detection and chemosensitivity of non-small cell lung cancer[J]. Cancer(English edition), 2011, 30(6): 407-414

[17] Berghauser Pont LM, Spoor JK, Venkatesan S, et al. The Bcl-2 inhibitor Obatoclax overcomes resistance to histone deacetylase inhibitors SAHA and LBH589 as radiosensitizers in patient-derived glioblastoma stem-like cells[J]. Genes Cancer, 2014, 5(11-12): 445-459

[18] Benavides-Serrato A, Anderson L, Holmes B, et al. mTORC2 modulates feedback regulation of p38 MAPK activity via DUSP10/MKP5 to confer differential responses to PP242 in glioblastoma[J]. Genes Cancer, 2014, 5(11-12): 393-406

[19] Kuo YC, Chen YC. Targeting delivery of etoposide to inhibit the growth of human glioblastoma multiforme using lactoferrin and folic acid-grafted poly(lactide-co-glycolide) nanoparticles[J]. Int J Pharm, 2015, 479(1): 138-149

[20] Sun Y, Guo F, Bagnoli M, et al. Key nodes of a microRNA network associated with the integrated mesenchymal subtype of high-grade serous ovarian cancer[J]. Chin J Cancer, 2015, 34(1): 28-40

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[42] Allaire E, Muscatelli-Groux B, Mandet C, et al. Paracrine effect of vascular smooth muscle cells in the prevention of aortic aneurysm formation[J]. J Vasc Surg, 2002, 36(5): 1018-1026

[43] McMillan WD, Patterson BK, Keen RR, et al. In situ localization and quantification of mRNA for 92-kD type IV collagenase and its inhibitor in aneurysmal,occlusive and normal aorta [J]. Arterioscler Thromb Vasc, Biol, 1995, 15(8): 1139-1144

[44] Ju X,Ijaz T, Sun H, et al. IL-6 Regulates Extracellular Matrix Remodeling Associated With Aortic Dilation in a Fibrillin-1 Hypomorphic mgR/mgR Mouse Model of Severe Marfan Syndrome [J]. J Am Heart Assoc, 2014, 3(1): e000476

[45] Pyo R, Lee JK, Shipley JM, et al. Targeted gene disruption of matrix metalloproteinase-9 (gelatine B) suppresses development of experimental abdominal aortic aneurysms[J]. J Clin Invest, 2000, 105 (11): 1641-1649

[46] Longo GM, Xiong W, Greiner TC, et al. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms [J]. J Clin Invest, 2002, 110(5): 625-632

[47] Kurihara T, Shimizu-Hirota R, Shimoda M, et al. Neutrophil-derived matrix metalloproteinase 9 triggers acute aortic dissection [J]. Circulation, 2012, 126(25): 3070-3080

[48] Segura AM, Luna RE, Horiba K, et al. Immunohistochemistry of matrix metalloproteinases and their inhibitors in thoracic aortic aneurysms and aortic valves of patients with Marfan's syndrome [J]. Circulation, 1998, 98 (19Suppl): 331-338

[49] Grigoryan A, Dimitrova A, Betova T, et al. P473Expressional changes of matrix metalloproteinases-2 and -9 in abdominal aorta in patients with atherosclerosis[J]. Cardiovasc Res, 2014, 103: 86-87

[50] Cheung YF, Hong WJ, Chan KW, et al. Modulating effects of matrix metalloproteinase-3 and -9 polymorphisms on aortic stiffness and aortic root dilation in patients after tetralogy of Fallot repair [J]. Int J Cardiol, 2011, 151(2): 214-217