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外泌体参与肺感染固有免疫过程的作用机制及其应用进展

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摘要 肺脏在保证机体与外界进行气体交换的过程中, 时刻面临着各种病原微生物、过敏原甚至是毒物的威胁。因此, 肺脏免疫系统, 尤其是固有免疫系统, 在保护机体免于上述危害并维持状态稳定中发挥着关键作用。面对外来病原体入侵, 肺泡上皮细胞(AECs)和肺泡巨噬细胞(AMs)相互协作, 迅速识别入侵病原体并发出招募信号, 进而中性粒细胞乃至适应性免疫细胞B细胞和T细胞迅速聚集浸润, 共同清除病原体, 维护机体健康。外泌体作为一种膜性功能单位, 来源于AECs和AMs等细胞, 包含蛋白质、DNA、miRNA、mRNA等物质, 能够有效地反映其来源细胞的状态, 因此可作为肺部疾病的生物标志物。此外, 外泌体包含物被靶细胞摄取后, 能够有效地传递信息, 调节靶细胞的细胞活动, 发挥治疗作用。该文主要就肺脏免疫系统的组成、肺感染固有免疫应答过程、外泌体在肺感染固有免疫应答中的角色和应用几个方面的研究进展进行综述。

关键词 肺脏; 感染; 固有免疫应答; 外泌体

Function of Exosome in Innate Immune Response during Pneumonia and Its Application

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Abstract In the daily gas exchange with the outside, the lung is always faced with the threat of various pathogenic microorganisms, allergens and even pollutants. Therefore, the lung immune system, especially the innate immune system, plays a key role in maintaining the homeostasis and protecting the body from the above hazards. When sufferring from the invasion of foreign pathogens, AECs (pulmonary epithelial cells) and AMs (alveolar macrophages) cooperate with each other to quickly identify the invading pathogens and send out recruitment signals, and then neutrophils and even adaptive immune cells like B cells and T cells, quickly gather and soak to jointly clear pathogens. As membranous units with specific function, exosome may come from AECs, AMs and other cells. They contain proteins, DNA, miRNA, mRNA and others, which can effectively reflect the state of its original cells and be used as biomarkers of lung diseases. In addition, exosome can also effectively transmit information and regulate biological activities when taken up by the target cells, which indicate its therapeutic role. Herein, this work mainly reviews the research progress in the lung immune system, the innate immune response in lung infection, and the role and application of exosome in pulmonary innate immune response during infection.

Keywords lung; infection; innate immune response; exosome

肺脏(lung)作为机体与外界进行气体交换的主要场所, 时刻面临着各种病原体、致敏物质或微尘的威胁, 也因而进化形成了其独特的免疫组成——肺脏免疫系统(pulmonary immune systerm), 以维持稳态和炎症之间的平衡[1-2]。肺脏免疫系统可分为固有免疫应答和获得性免疫应答; 固有免疫应答无需“初次免疫”, 负责迅速且广泛地应对外来入侵, 而获得性免疫应答则需一定的时间(数天至数周)方至完全反应状态, 具有特异性和记忆性, 能够在入侵物再次暴露时形成更为强大的免疫反应; 两者相辅相成, 共同保护着肺脏健康[1]。肺脏固有免疫系统通过识别病原体相关分子模式(pathogen-associated molecular patterns, PAMPs)或微生物相关分子模式(microbe associated molecular patterns, MAMPs), 成为抵御外来病原体的第一道防线。近年来, 外泌体在免疫过程中的角色已被广泛研究[3]。外泌体所携带的蛋白、代谢物和核酸等物质被其靶细胞摄取后, 能够有效地改变它们的生物活动, 促进或抑制疾病进展, 因而外泌体在许多疾病(如感染、退行性疾病或癌症)的发生发展中起到了重要作用。此外, 修饰后外泌体被报道能够激发获得性免疫应答, 其在感染和恶性肿瘤中的免疫协调功能和治疗作用得到进一步的证实。外泌体的免疫调节功能可能是通过抗原肽的转运和提呈、DNA诱导cGAS-STING信号通路的传输、所携带的miRNA对基因表达的调控和膜表面配体对不同信号通路的触发所实现的[4]。肺脏作为具有特殊免疫系统的器官, 其众多生理病理过程中必然存在着外泌体的身影, 也是外泌体应用的重要潜在部位。近日, 有临床试验尝试利用外泌体将外源性CD24蛋白注入COVID-19患者的肺部, 以预防细胞因子风暴过度反应, 保证患者免疫系统有效杀灭病毒, 目前有研究在I期临床试验中成功治愈了30位感染者中的29人, 治愈率超过95%(https://clinicaltrials.gov/ct2/show/NCT04747574)。可见, 外泌体可能参与肺脏感染免疫过程的许多活动, 反映该过程不同阶段的物质变化, 也影响该过程的最终结局。在这里, 我们着重综述外泌体在肺脏感染固有免疫反应过程中的作用和参与机制, 为外泌体在肺脏感染诊断和治疗中的应用提供资料。

1 肺感染固有免疫过程

1.1 肺脏——免疫器官

肺脏是机体的重要器官之一, 不仅承担着气体交换的任务, 而且具有重要的免疫功能, 保护机体免受呼吸过程中吸入的病原体、过敏原或有毒有害物质的侵害[5]。从生理学和免疫学的角度来看, 肺可分为两部分: (1) 上呼吸道黏膜(IgA抗体为主)和腺体部分, (2) 非黏膜性外周气道组织(以IgG抗体为主)。此外, 在肺泡腔内还驻守着肺泡巨噬细胞(占90%)和淋巴细胞(占10%)。也就是说, 肺免疫系统由不同部分组成, 各部分之间能够相互作用, 共同完成生理性和病理性免疫活动[6]。

如同肠道黏膜的淋巴组成, 呼吸道黏膜中也存在着淋巴细胞, 发挥着抵御外界病原体入侵的作用。此外, 呼吸道支气管壁内的固有淋巴组织中还存在着呼吸淋巴样细胞(respiratory lymphoid cells, RLCs),

如孤立淋巴滤泡(solitary lymphoid follicles, SLFs)或小肠Peyer淋巴结(Peyer's patches, PPs)。因此, 呼吸道相关淋巴样组织(bronchus-associated lymphoid tissue, BALT)在形态和功能上都类似于小肠的肠道相关淋巴样组织(gut-associated lymphoid tissue, GALT)。BALT被淋巴上皮覆盖, 其滤泡相关上皮能够选择性地感知呼吸道腔内的可溶性和颗粒性物质。值得一提的是, 在人体内, BALT存在于儿童和青少年的肺部, 而只有在慢性炎症性疾病发生时才见于成人, 即诱导型BALT(iBALT)。此外, BALT可能存在于胎儿和新生儿的肺部, 且取决于抗原刺激[7]。然而, 当成人因支气管或支气管肺泡阻塞而反复发生不明原因的呼吸道感染时, 包括淋巴样滤泡在内的RLCs就会显著扩增。B细胞也是BALT的重要组成之一, 主要负责产生分泌IgA。IgA可与淋巴细胞结合, 增强其抗体依赖的细胞毒作用, 还可以保护机体免于过敏反应时的病毒或细菌感染。BALT还包括了T细胞区, 其中也有树突状细胞(dendritic cells, DCs)存在。另外, BALT富有高内皮静脉(high endothelial venule, HEV), 以便于淋巴细胞和抗原循环。同时, 支气管肺泡细胞(broncho-alveolar cells, BACs)也存在于肺脏内, 可经外周气道的支气管肺泡灌洗液(broncho-alveolar lavage fluid, BALF)获取。在健康状态下, BALF中的BACs主要包含AMs(约占90%)和淋巴细胞(约占10%)[8], 负责防御外来病原体、有毒物质和过敏原等, 调节肺脏固有免疫和获得性免疫过程。

出生后, 肺免疫系统在与环境的相互作用中逐渐成熟, 主要依赖于暴露抗原的丰富程度和类型[9]。然而, 肺在子宫内胚胎发育期间是保持无菌的, 经阴道分娩时才获得了母体的微生物群[10-11]。肺微生物群有助于肺免疫系统的发育、免疫耐受的诱导及其内环境稳定[12-13]。肺上皮细胞、ILCs、AMs和其他肺免疫细胞, 在肺免疫稳态的维持中发挥着必不可少的作用。然而, 它们也会在识别病原体或过敏原后, 引起肺部炎症改变。在某些情况下, 这些肺部炎症变化是轻微的并且能够自发缓解, 但一旦发展为急性肺损伤/急性呼吸窘迫综合征(acute lung injury/acute respiratory distress syndrome, ALI/ARDS), 则可能对宿主有害, 这取决于感染的严重程度和固有免疫反应状态[8]。

1.2 肺感染固有免疫应答

肺感染是一类严重威胁人类健康的疾病, 尤其对于儿童和老年人, 是由病原微生物入侵下呼吸道后引发的复杂的病理生理过程[14]。例如, 肺炎链球菌(*S. pneumoniae*)就是社区获得性肺炎(community-acquired pneumonia, CAP)最常见的病原体, 此外, 嗜肺军团菌(*Legionella pneumophila*)、肺炎支原体(*Mycoplasma pneumoniae*)、肺炎衣原体(*Chlamydophila pneumoniae*)、鹦鹉热衣原体(*Chlamydophila psittaci*)、伯纳特氏立克次体(*Coxiella burnetii*)也是常见的CAP病原体[8]。绝大多数医院获得性肺炎(hospital-acquired pneumonia, HAP)则是由革兰氏阴性菌如[肺炎克雷伯菌(*Klebsiella pneumoniae*)、铜绿假单胞菌(*Klebsiella pneumoniae*)等]引发的[14]。肺感染固有免疫应答始于固有免疫细胞如AECs和AMs等的激活, 继而招募中性粒细胞浸润。文献报道, Toll样受体4(toll-like receptor 4, TLR4)激活会引发免疫应答, 改善革兰氏阳性菌(肺炎链球菌)或革兰氏阴性菌(肺炎克雷伯菌)肺炎小鼠的生存状况[15]。

1.2.1 气道上皮细胞 气道上皮细胞构成了抵御外来病原体的机械性屏障, 主要包括支气管上皮细胞(bronchial epithelial cells, BECs)和肺泡上皮细胞(alveolar epithelial cells, AECs), 后者又分为I型和II型, I型负责气体交换和病原体识别, II型则负责产生分泌肺表面活性物质, 也是一类固有免疫细胞[16-17]。II型AECs还能够向基底膜外侧分泌修复酶(如纤维蛋白原), 迅速改善细胞渗透压, 识别病原体释放的穿孔毒素, 参与炎症细胞损伤时的细胞应答。外界病原体入侵时, AECs能够释放免疫分子如抗微生物多肽(antimicrobial peptides, AMPs)和细胞因子, 进而调节初始免疫应答, 还能够与AMs、中性粒细胞和DCs等直接相互作用[18]。持续的AECs激活将释放大量的促炎因子和趋化因子, 甚至促进细胞死亡(坏死或焦亡), 因而可能对宿主有害。

AECs表达多种模式识别受体(pattern recognition receptor, PRRs), 如Toll 样受体(toll-like receptors, TLRs)(胞膜TLRs如TLR1、TLR2、TLR4、TLR5、TLR6, 胞内TLRs如TLR3、TLR7、TLR8、TLR9)和炎症小体在内的NLRs, 能够迅速有效地识别各种病原微生物[17,19-21], 成为防御和清除病原微生物的第一道防线。TLRs通路的下游接头蛋白[髓样分化蛋白抗原88(MyD88)]和诱导IFN-β的Toll/IL-1受体结构域接头分子(TIR-domain-containing adapter-inducing

interferon-β, TRIF)在细菌性肺炎的发生与发展中起到关键作用。例如, MyD88$^{-/-}$和TRIF$^{-/-}$的革兰氏阴性菌(肺炎克雷伯菌、铜绿假单胞菌和大肠杆菌)肺炎小鼠体内Th1免疫应答(TNFα、IL-6和IL-8)减弱, 中性粒细胞浸润减少, 活化T细胞表达和分泌产物(RANTES或CCL5)受到影响, 最终表现出严重的症状体征[22-25]。在肺炎克雷伯菌肺炎中, TLRs被激活后启动TRIF通路, 促进IFN-x03B3的表达, 进而产生抗菌作用[26]。然而, Toll/IL-1受体结构域接头蛋白(toll/interleukin-1 receptor domain containing adaptor protein, TIRAP)在铜绿假单胞菌肺炎中却并未起到关键作用, 因为LIX分泌、中性粒细胞浸润和菌体清除并不依赖TIRAP[27]。

1.2.2 肺巨噬细胞 稳态下, 肺巨噬细胞占肺脏所有免疫细胞的90%~95%[28]。肺巨噬细胞可分为两类: 间质巨噬细胞(interstitial macrophages, IMs), 主要位于肺脏间质内, 分子标记为CD11b^{+}CD11c^{low}, AMs主要位于肺泡腔内, 分子标记为CD11c^{+}CD11b^{low}[29], 未激活状态下表达CD206和β葡聚糖特异性受体Dectin-1[30-31]。AMs在肺脏免疫稳态的维持和免疫防御中扮演着重要角色, 而IMs则在其中发挥调节作用[32]。

在肺感染和ALI/ARDS的发生发展过程中, AMs所发挥的免疫作用因病原体种类和疾病进程而异。肺炎链球肺炎时, 循环单核−巨噬细胞浸润, AMs负责清除凋亡细胞, 但其清除病原体能力相对减弱, 从而导致肺内病原体负荷加重[33-34]。G^{-}菌入侵时, AMs能够释放TNFα, 诱导AECs产生粒细胞−巨噬细胞集落刺激因子(granulocyte-macrophage colony-stimulating factor, GM-CSF), 并通过旁分泌形式促进AECs增殖以修复肺脏机械性屏障[35]。随着M1型巨噬细胞向M2型的极化, 肺感染进入恢复期, 这主要依赖M2型巨噬细胞分泌并释放IL-4和IL-13进而激活STAT6通路实现[36]。这一过程中, AMs的吞噬能力下降, 可能导致机体出现严重的二次肺感染。而且, 研究发现, 正常小鼠AMs被移植入恢复期肺炎小鼠肺内后, 其吞噬能力也出现下降, 证实机体在肺感染恢复期存在肺脏固有免疫抑制状态[37], 且与信号调节蛋白α(signal regulatory protein α, SIRPα)的高表达密切相关[38-39], 以保证肺脏炎症反应的缓解。

1.2.3 AECs与AMs之间的相互作用 目前认为, AECs与AMs间的相互作用主要起到抑炎作用。AECs的膜表面表达的CD200, 能够与AMs膜表面的CD200R相结合, 以抑制其促炎活动, 避免肺感染进展为ALI/ARDS; 而CD200R在AMs的高表达依赖于AECs产生并分泌的IL-10和TGF-β[40]。CD200缺失小鼠在肺感染时容易出现炎症因子风暴甚至进展为ALI/ARDS[40]。CD200-CD200R相互作用还能够通过C/EBP-β通路维持M2a表型, 上调TGF-β的表达水平[41]。研究发现, CD200-CD200R相互作用能够激活JAK/STAT-1通路, 进而抑制ERK1/2通路, 最终抑制巨噬细胞激活[42]。此外, AMs在肺感染时还能够与AECs形成Cx43(connexin 43)缝隙连接通道, 从而通过同步Ca^{2+}流达到信息交互的目的[43-44]。可见, AECs与AMs的相互作用影响着肺感染时炎症反应的结局, 避免肺感染进展为ALI/ARDS, 促进炎症反应缓解和机体修复。

1.2.4 中性粒细胞 不同病原体所致的肺感染和其相关ALI/ARDS, 中性粒细胞浸润的机制不尽相同。研究发现, G^{+}菌(如肺炎链球菌)感染时, CD29或β1整合素参与介导中性粒细胞浸润, 而G^{-}菌(如大肠杆菌*E.coli*或铜绿假单胞菌)感染时, 该过程则由CD18或β2整合素诱导[45]。也有文献报道, ALI患者BALF中的趋化因子(如CXCL8、IL-8、CXCL1、KC、CXCL5、CCL2及ENA-78)的水平明显升高, 可能参与调节中性粒细胞的浸润过程[46]。

中性粒细胞的膜表面表达趋化受体CXCR2和FPRs, 能够与多种趋化因子(如CXCL1、CXCL8、CXCL5、CXCL2、CXCL3、CXCL6、CXCL7等)结合, 以调节中性粒细胞向肺部浸润[47-48]。有趣的是, 在*E.coli*诱导的肺炎小鼠的肺内, CXCL5缺失使得中性粒细胞浸润增多, 有助于机体清除病原体, 减轻肺水肿, 避免重症肺炎或ALI/ARDS的发生; 而且, CXCL5缺失小鼠的骨髓和外周血中性粒细胞CXCR2的表达水平并无显著改变[49]。CAI等[50]研究发现, 在肺炎克雷伯菌感染肺时, CXCL1通过调节CXCL2/MIP2和CXCL5的水平, 激活NF-κB和MAPKs通路, 进而影响中性粒细胞浸润和病原体清除过程。可见, 肺部中性粒细胞浸润与肺感染结局有着密切关联。

1.2.5 固有淋巴样细胞 作为调节性固有免疫细胞存在的固有淋巴样细胞(ILCs), 在肺部炎症和炎症性疾病的发生发展中亦发挥重要的作用[51-52]。ILCs主要分为ILC1s和自然杀伤细胞(NK cells)、

ILC2s和ILC3s[53], 在肺脏内均有分布[54]。ILC1s和NK细胞能够迅速释放IFN-γ; 在肺炎克雷伯菌或嗜肺军团菌肺炎小鼠中, IFN-γ缺失会影响 IL-1和IL-6的产生, 从而降低机体清除病原体的能力, 导致重症肺炎的发生[55-56]。ILC2s主要产生Th2因子(IL-4、IL-5、IL-6、IL-9、IL-13和Arg), 而ILC3s主要释放IL-17、IL-22、人IL-26、GM-CSF和TNFα[57]。ILC2s能够向ILC1s转化以清除病原体, 还参与IL-13依赖的M2巨噬细胞极化, 影响着肺感染结局[58]。研究发现, ILC3s在肺炎链球菌肺炎发生时能够迅速聚集, 释放IL-22, 参与肺脏DCs的激活, 保护机体免于重症肺炎或ALI/ARDS[59]; 在肺炎克雷伯菌肺炎发生时, ILC3s则能够释放IL-17, 有助于最终的炎症消散[60]; 此外, ILC3s还能够释放IL-17A, 增强肺巨噬细胞的吞噬和杀伤能力, 促进机体清除病原体[61]。综合而言, ILCs在肺感染固有免疫应答中起到重要作用, 能够促进炎症消散, 保护机体免于ALI/ARDS。

1.2.6 细胞因子和趋化因子 肺感染免疫应答中, 各类免疫细胞表达并释放细胞因子(如IFN-γ、TNFα、IL-1和IL-6等), 以达到胞间信息互换目的, 尤其是在病程早期的促炎阶段[1]。此外, IL-10也是颇受关注的细胞因子, 它能够影响单核−巨噬细胞的MHC II的表达和细胞因子产生, 进而影响T细胞的增殖和细胞因子生成、NK细胞的细胞因子产生和B细胞免疫球蛋白的分泌等活动[62]。

趋化因子多是8~10 kDa大小的糖蛋白, 通过与G蛋白偶联受体(G protein coupled receptors, GPCRs)结合传递信息[63], 主要功能是调节白细胞迁移和活化[64]。基于氨基酸结构, 趋化因子可被分为四类, 其中最为主要的是CC趋化因子(如MCP-1、MIP-1α和RANTES, 负责趋化单核细胞、淋巴细胞、嗜碱性粒细胞和嗜酸性粒细胞)和CXC趋化因子(如 IL-8、GRO-α和ENA-78, 负责趋化中性粒细胞)。IL-8是一种非常重要的中性粒细胞趋化和活化因子, 主要由肺内皮和上皮细胞受到LPS、IL-1β或TNFα刺激后产生, 作用于趋化受体CXCR1和CXCR2, 在多种急慢性肺部疾病均发挥作用。CKLF1是一种人趋化因子, 北京大学医学部王应教授组[65]曾对其做过系列研究和报道, 他们首先发现CKLF1能够有效地趋化人白细胞, 其氨基酸序列与TARC/CCL17和MDC/CCL22具有相似性, 并通过PTX阻断实验、钙流实验和受体内化实验证实其作用受体为CCR4; 随后, 他们进一步发现和证实CKLF1的C-端肽C27和C19在其与CCR4相互作用中发挥关键作用[66]; C27和C19能够抑制SDF-1作用于CXCR4的趋化功能和CXCR4内化, 其中机制主要是两种肽作用于CCR4后激活PI3K/PKC通路, 进而使得CXCR4脱敏[67]; 经鼻腔或腹腔给予过敏性鼻炎小鼠后, C19能够有效地降低血浆IgE水平, 抑制鼻内黏膜和肺组织中的嗜酸性粒细胞聚集, 降低局部鼻腔组织中的Th2细胞因子IL-4, 从而减轻过敏症状[68]。可见, 趋化因子在肺部炎症性疾病中的重要信使, 也影响着疾病结局和转归。

2 外泌体与肺感染固有免疫应答

2.1 外泌体

外泌体(exosome)是由活细胞分泌的直径为50~150 nm的胞外囊泡(extracellular vesicles, EVs)。目前认为, 外泌体的形成和释放存在两种方式, 即内体分选转运复合体(endosomal sorting complex required for transport, ESCRT)依赖和ESCRT非依赖形式[69-70]。不同细胞来源的外泌体所包含的物质有所不同, 但大致可分为蛋白质、脂质和核酸[4]。典型的外泌体具有与其来源细胞相同的磷脂双分子层膜结构[71], 具有丰富的胆固醇、鞘磷脂、神经酰胺和去污剂耐受膜结构域(即脂阀)[72], 以及脂阀相关蛋白, 如糖基磷脂酰肌醇锚连膜蛋白和脂阀结构蛋白[73]。外泌体的特征性蛋白包括ESCRT复合体组成蛋白如Alix、肿瘤易感基因101(tumor susceptibility gene 101, TSG101)[74-75]以及四跨膜蛋白如CD9、CD63、CD81和CD82[76]; 其他蛋白组分还包括参与外泌体的组装和膜融合过程的胞浆蛋白如Rabs, 以及参与调节胞膜骨架变化和膜融合过程的膜联蛋白等[77]。此外, 外泌体中包裹着多种多样的核酸物质, 包括非编码RNAs如microRNA、lncRNA、tRNA片段、siRNAs、结构RNAs、小RNA转录本、RNA-蛋白复合体和DNA如染色体DNA、线粒体DNA等[78-79]。研究发现, 外泌体参与着免疫反应、病毒感染、怀孕、心血管疾病、中枢神经系统疾病和恶性肿瘤等众多生命活动[77]。

2.2 外泌体与感染免疫

外泌体能够在免疫应答中传递和提呈抗原肽、cGAS-STING的诱导DNA、miRNA和一些表面配体等, 以发挥调节作用[4]。研究发现, APCs来源的外泌

体可携带p-MHC II和共刺激分子, 从而直接将抗原肽提呈给特定T细胞并将其激活[80]。此外, 细菌(如结核分枝杆菌、幽门螺杆菌)感染时, 巨噬细胞来源的外泌体也能够通过提呈抗原肽增强免疫应答, 并进一步影响适应性免疫应答[81]; 而且, 巨噬细胞来源的外泌体还包含有IFN-α、IFN-γ、TNFα和白细胞介素(IL), 能够促进DCs成熟, 激活$CD4^+$和$CD8^+$T细胞[82], 甚至调节巨噬细胞IL的表达[83]。外泌体中包含的DNA和miRNA, 如一些胞内菌(如李斯特菌、嗜肺军团菌、土拉弗朗西斯菌)DNA, 也参与调节和适应性免疫应答。有研究报道, 在结核分枝杆菌感染过程中, 巨噬细胞来源的外泌体中含有核分枝杆菌RNA, 能够激活RNA感受通路, 增强其他巨噬细胞的吞噬能力, 促进免疫应答[84]。然而, 在李斯特菌感染时, 包含有李斯特菌DNA的外泌体却发挥T细胞抑制功能, 以减弱机体杀菌能力[85]。而关于外泌体在真菌或寄生虫感染中的作用, 目前研究尚少。通过传递miRNA, 外泌体还能够影响摄取细胞的基因表达和信号通路, 从而发挥免疫调节作用。DCs胞间通过外泌体递送miRNA, 抑制基因表达, 影响细胞成熟[86]。文献报道, 外泌体参与多种病毒感染过程。外泌体能够为病毒提供“假性外壳”, 帮助病毒扩散[87]。当然, 外泌体也参与抗病毒免疫过程。例如, 经IFN-α刺激后巨噬细胞来源的外泌体膜表达抗病毒成分APOBEC3G, 以帮助机体抵御乙型肝炎病毒(HBV)[88]或人类免疫缺陷病毒(HIV-1)[89]的侵入。

2.3 外泌体在肺感染和ALI/ARDS固有免疫应答中的角色

近年来, 包括外泌体在内的EVs在ALI/ARDS中的作用已得到广泛研究[90]。首先, 外泌体在肺脏免疫稳态的维持中发挥着一定的作用。MATTHAY等[90]研究发现, AECs来源的外泌体含有能够中和人流感病毒的黏蛋白(MUC)-1、MUC-4、MUC-16, 以保护机体免于该病毒的入侵。同时, AECs来源的外泌体miR-92a-3p能够激活肺泡巨噬细胞, 进而参与ALI的发生与发展过程[91]。研究发现, 在LPS诱发的ALI的发生发展过程中, 肺泡灌洗液中的外泌体首先来自于肺泡巨噬细胞, 这些外泌体中含有许多的促炎因子, 进而刺激中性粒细胞释放更多的促炎因子和IL-10, 而IL-10则促进肺泡巨噬细胞向M2型极化, 参与肺组织修复和纤维化[92]。还有研究报道, 肺泡巨噬细胞来源的外泌体中包含细胞因子信号抑制物(suppressors of cytokine signaling, SOCS)-1和SOCS-3, 能够抑制AECs的STAT通路, 减轻肺部炎症[93]。检测无菌性刺激后BALF中的EVs, 结果发现TLR2、IL-6、TNFα和Myd88水平均显著升高; 而在感染性因素刺激下, 则发现TLR6、IL-1β和IL-10水平显著升高[94-95]。有研究发现, 感染结核分枝杆菌的巨噬细胞来源的外泌体中含有结核分枝杆菌的转录本, 其中的miRNA丰富度下降, 但存在超过100种miRNA是特异性的, 这些外泌体来源的miRNA被未活化的巨噬细胞摄取后, 能够诱导其释放炎症因子或启动凋亡[96]。中性粒细胞来源的外泌体, 也被报道参与慢性阻塞性肺疾病(chronic obstructive pulmonary disease, COPD)和支气管肺发育不良(bronchopulmonary dysplasia, BPD)的病理发展过程[97]。可见, AECs或固有免疫细胞来源的外泌体在肺脏免疫应答过程中发挥着巨大的作用。

2.4 外泌体在肺感染和ALI/ARDS中的应用

包括外泌体在内的EVs-miRNAs、外泌体蛋白等, 能够反映其来源细胞的状态和功能, 是非常有潜力的生物标志物[78,98], 目前对于肺癌展现出了良好的预测和诊断价值[99-100], 而肺感染和ALI/ARDS相关机制仍待更多的探索。近期研究提示, 间充质干细胞来源的外泌体可能通过抑制促炎因子的释放、携带PEG2而诱导M2型巨噬细胞极化, 促进抑炎因子如IL-10的分泌, 产生KGF、VEGF和HGF等促进损伤组织修复等, 进而对SARS-CoV-2感染起到治疗作用[101-102], 相关临床试验(NCT04276987、NCT0438938、NCT04384445、ChiCTR2000030484、ChiCTR2000030261)目前正在招募进行中。此外, 包括外泌体在内的EVs稳定、能够有效保护运载物且易被靶细胞摄取, 也十分有希望成为新型药物载体[103-104]。其中, 外泌体作为miRNA载体研究较多。ZHANG等[105]尝试改进氯化钙转染方法, 从而直接将miRNA转入至外泌体中, 并通过体内和体外实验验证了其有效性, 为外泌体作为药物载体经气管给药治疗肺部疾病提供了可靠证据。前文曾述, $CD24^+$外泌体在COVID-19的治疗中展现出可喜疗效。因此, 外泌体调节肺感染免疫应答将在未来得到更多的发展和应用。

3 总结

肺脏是机体重要的免疫器官之一, 固有免疫系

统在其稳态的维持和外来病原体的清除中都发挥着极为重要的作用。毫无疑问, 深入认识肺脏固有免疫防御的分子机制, 将为临床提供新的干预靶点。近些年来, 外泌体在肺脏固有免疫应答中的作用和机制得到不断揭示。将外泌体经呼吸道应用的尝试, 有望开启肺感染治疗的新局面。当然, 不论是用作疾病的生物标记物, 还是用于治疗, 仍需更多研究以确保外泌体应用于临床的可靠性和安全性。

参考文献 (References)

[1] SUZUKI T, CHOW C W, DOWNEY G P. Role of innate immune cells and their products in lung immunopathology [J]. Int J Biochem Cell Biol, 2008, 40(6/7): 1348-61.

[2] HASENBERG M, STEGEMANN-KONISZEWSKI S, GUNZER M. Cellular immune reactions in the lung [J]. Immunol Rev, 2013, 251(1): 189-214.

[3] ROBBINS P D, MORELLI A E. Regulation of immune responses by extracellular vesicles [J]. Nat Rev Immunol, 2014, 14(3): 195-208.

[4] XUNIAN Z, KALLURI R. Biology and therapeutic potential of mesenchymal stem cell-derived exosomes [J]. Cancer Sci, 2020, 111(9): 3100-10.

[5] KUMAR V, CHHIBBER S. Acute lung inflammation in Klebsiella pneumoniae B5055-induced pneumonia and sepsis in BALB/c mice: a comparative study [J]. Inflammation, 2011, 34(5): 452-62.

[6] MCDERMOTT M R, BEFUS A D, BIENENSTOCK J. The structural basis for immunity in the respiratory tract [J]. Int Rev Exp Pathol, 1982, 23: 47-112.

[7] ERSCH J, TSCHERNIG T, STALLMACH T. Frequency and potential cause of bronchus-associated lymphoid tissue in fetal lungs [J]. Pediatr Allergy Immunol, 2005, 16(4): 295-8.

[8] KUMAR V. Pulmonary innate immune response determines the outcome of inflammation during pneumonia and sepsis-associated acute lung injury [J]. Front Immunol, 2020, 11: 1722.

[9] LLOYD C M, MARSLAND B J. Lung homeostasis: influence of age, microbes, and the immune system [J]. Immunity, 2017, 46(4): 549-61.

[10] GALLACHER D J, KOTECHA S. Respiratory microbiome of new-born infants [J]. Front Pediatr, 2016, 4: 10.

[11] DOMINGUEZ-BELLO M G, COSTELLO E K, CONTRERAS M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns [J]. Proc Natl Acad Sci USA, 2010, 107(26): 11971-5.

[12] GOLLWITZER E S, SAGLANI S, TROMPETTE A, et al. Lung microbiota promotes tolerance to allergens in neonates via PD-L1 [J]. Nat Med, 2014, 20(6): 642-7.

[13] WANG J, LI F, SUN R, et al. Bacterial colonization dampens influenza-mediated acute lung injury via induction of M2 alveolar macrophages [J]. Nat Commun, 2013, 4: 2106.

[14] CILLONIZ C, MARTIN-LOECHES I, GARCIA-VIDAL C, et al. Microbial etiology of pneumonia: epidemiology, diagnosis and resistance patterns [J]. Int J Mol Sci, 2016, 17(12): 2120.

[15] BRANGER J, KNAPP S, WEIJER S, et al. Role of toll-like receptor 4 in gram-positive and gram-negative pneumonia in mice [J]. Infect Immun, 2004, 72(2): 788-94.

[16] EISELE N A, ANDERSON D M. Host defense and the airway epithelium: frontline responses that protect against bacterial invasion and pneumonia [J]. J Pathog, 2011, 2011: 249802.

[17] LEIVA-JUAREZ M M, KOLLS J K, EVANS S E. Lung epithelial cells: therapeutically inducible effectors of antimicrobial defense [J]. Mucosal Immunol, 2018, 11(1): 21-34.

[18] HIPPENSTIEL S, OPITZ B, SCHMECK B, et al. Lung epithelium as a sentinel and effector system in pneumonia-molecular mechanisms of pathogen recognition and signal transduction [J]. Respir Res, 2006, 7: 97.

[19] GAY N J, SYMMONS M F, GANGLOFF M, et al. Assembly and localization of Toll-like receptor signalling complexes [J]. Nat Rev Immunol, 2014, 14(8): 546-58.

[20] CHAPUT C, SANDER L E, SUTTORP N, et al. NOD-like receptors in lung diseases [J]. Front Immunol, 2013, 4: 393.

[21] LEE S, SUH G Y, RYTER S W, et al. Regulation and function of the nucleotide binding domain leucine-rich repeat-containing receptor, pyrin domain-containing-3 inflammasome in lung disease [J]. Am J Respir Cell Mol Biol, 2016, 54(2): 151-60.

[22] CAI S, BATRA S, SHEN L, et al. Both TRIF- and MyD88-dependent signaling contribute to host defense against pulmonary Klebsiella infection [J]. J Immunol, 2009, 183(10): 6629-38.

[23] VAN LIESHOUT M H, BLOK D C, WIELAND C W, et al. Differential roles of MyD88 and TRIF in hematopoietic and resident cells during murine gram-negative pneumonia [J]. J Infect Dis, 2012, 206(9): 1415-23.

[24] POWER M R, LI B, YAMAMOTO M, et al. A role of Toll-IL-1 receptor domain-containing adaptor-inducing IFN-beta in the host response to Pseudomonas aeruginosa lung infection in mice [J]. J Immunol, 2007, 178(5): 3170-6.

[25] JEYASEELAN S, YOUNG S K, FESSLER M B, et al. Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF)-mediated signaling contributes to innate immune responses in the lung during *Escherichia coli* pneumonia [J]. J Immunol, 2007, 178(5): 3153-60.

[26] VAN LIESHOUT M H, FLORQUIN S, VAN'T VEER C, et al. TIR-domain-containing adaptor-inducing interferon-beta (TRIF) mediates antibacterial defense during gram-negative pneumonia by inducing interferon-x03B3 [J]. J Innate Immun, 2015, 7(6): 637-46.

[27] JEYASEELAN S, YOUNG S K, YAMAMOTO M, et al. Toll/IL-1R domain-containing adaptor protein (TIRAP) is a critical mediator of antibacterial defense in the lung against *Klebsiella pneumoniae* but not *Pseudomonas aeruginosa* [J]. J Immunol, 2006, 177(1): 538-47.

[28] KOPF M, SCHNEIDER C, NOBS S P. The development and function of lung-resident macrophages and dendritic cells [J]. Nat Immunol, 2015, 16(1): 36-44.

[29] BYRNE A J, MATHIE S A, GREGORY L G, et al. Pulmonary macrophages: key players in the innate defence of the airways [J]. Thorax, 2015, 70(12): 1189-96.

[30] DAVIES L C, JENKINS S J, ALLEN J E, et al. Tissue-resident macrophages [J]. Nat Immunol, 2013, 14(10): 986-95.

[31] TAYLOR P R, MARTINEZ-POMARES L, STACEY M, et al.

Macrophage receptors and immune recognition [J]. Annu Rev Immunol, 2005, 23: 901-44.
[32] BYRNE A J, MAHER T M, LLOYD C M. Pulmonary macrophages: a new therapeutic pathway in fibrosing lung disease [J]? Trends Mol Med, 2016, 22(4): 303-16.
[33] TAUT K, WINTER C, BRILES D E, et al. Macrophage turnover kinetics in the lungs of mice infected with streptococcus pneumoniae [J]. Am J Respir Cell Mol Biol, 2008, 38(1): 105-13.
[34] MEDEIROS A I, SEREZANI C H, LEE S P, et al. Efferocytosis impairs pulmonary macrophage and lung antibacterial function via PGE2/EP2 signaling [J]. J Exp Med, 2009, 206(1): 61-8.
[35] CAKAROVA L, MARSH L M, WILHELM J, et al. Macrophage tumor necrosis factor-alpha induces epithelial expression of granulocyte-macrophage colony-stimulating factor: impact on alveolar epithelial repair [J]. Am J Respir Crit Care Med, 2009, 180(6): 521-32.
[36] NEPAL S, TIRUPPATHI C, TSUKASAKI Y, et al. STAT6 induces expression of Gas6 in macrophages to clear apoptotic neutrophils and resolve inflammation [J]. Proc Natl Acad Sci USA, 2019, 116(33): 16513-8.
[37] ROQUILLY A, MCWILLIAM H E G, JACQUELINE C, et al. Local modulation of antigen-presenting cell development after resolution of pneumonia induces long-term susceptibility to secondary infections [J]. Immunity, 2017, 47(1): 135-47,e135.
[38] BARCLAY A N, BROWN M H. The SIRP family of receptors and immune regulation [J]. Nat Rev Immunol, 2006, 6(6): 457-64.
[39] VEILLETTE A, CHEN J. SIRPalpha-CD47 immune checkpoint blockade in anticancer therapy [J]. Trends Immunol, 2018, 39(3): 173-84.
[40] HEROLD S, MAYER K, LOHMEYER J. Acute lung injury: how macrophages orchestrate resolution of inflammation and tissue repair [J]. Front Immunol, 2011, 2: 65.
[41] HAYAKAWA K, WANG X, LO E H. CD200 increases alternatively activated macrophages through cAMP-response element binding protein-C/EBP-beta signaling [J]. J Neurochem, 2016, 136(5): 900-6.
[42] OCANA-GUZMAN R, VAZQUEZ-BOLANOS L, SADA-OVALLE I. Receptors that inhibit macrophage activation: mechanisms and signals of regulation and tolerance [J]. J Immunol Res, 2018, 2018: 8695157.
[43] WESTPHALEN K, GUSAROVA G A, ISLAM M N, et al. Sessile alveolar macrophages communicate with alveolar epithelium to modulate immunity [J]. Nature, 2014, 506(7489): 503-6.
[44] SARIEDDINE M Z, SCHECKENBACH K E, FOGLIA B, et al. Connexin43 modulates neutrophil recruitment to the lung [J]. J Cell Mol Med, 2009, 13(11/12): 4560-70.
[45] MIZGERD J P. Molecular mechanisms of neutrophil recruitment elicited by bacteria in the lungs [J]. Semin Immunol, 2002, 14(2): 123-32.
[46] GOODMAN R B, STRIETER R M, MARTIN D P, et al. Inflammatory cytokines in patients with persistence of the acute respiratory distress syndrome [J]. Am J Respir Crit Care Med, 1996, 154(3 Pt 1): 602-11.
[47] MAAS S L, SOEHNLEIN O, VIOLA J R. Organ-specific mechanisms of transendothelial neutrophil migration in the lung, liver, kidney, and aorta [J]. Front Immunol, 2018, 9: 2739.
[48] DORWARD D A, LUCAS C D, CHAPMAN G B, et al. The role of formylated peptides and formyl peptide receptor 1 in governing neutrophil function during acute inflammation [J]. Am J Pathol, 2015, 185(5): 1172-84.
[49] MEI J, LIU Y, DAI N, et al. CXCL5 regulates chemokine scavenging and pulmonary host defense to bacterial infection [J]. Immunity, 2010, 33(1): 106-17.
[50] CAI S, BATRA S, LIRA S A, et al. CXCL1 regulates pulmonary host defense to Klebsiella Infection via CXCL2, CXCL5, NF-kappaB, and MAPKs [J]. J Immunol, 2010, 185(10): 6214-25.
[51] MAI K, CHUI J J, DI GIROLAMO N, et al. Role of toll-like receptors in human iris pigment epithelial cells and their response to pathogen-associated molecular patterns [J]. J Inflamm, 2014, 11: 20.
[52] KUMAR V. Innate lymphoid cells: new paradigm in immunology of inflammation [J]. Immunol Lett, 2014, 157(1/2): 23-37.
[53] DIEFENBACH A, COLONNA M, KOYASU S. Development, differentiation, and diversity of innate lymphoid cells [J]. Immunity, 2014, 41(3): 354-65.
[54] BARLOW J L, MCKENZIE A N J. Innate lymphoid cells of the lung [J]. Annu Rev Physiol, 2019, 81: 429-52.
[55] MOORE T A, PERRY M L, GETSOIAN A G, et al. Divergent role of gamma interferon in a murine model of pulmonary versus systemic *Klebsiella pneumoniae* infection [J]. Infect Immun, 2002, 70(11): 6310-8.
[56] SHINOZAWA Y, MATSUMOTO T, UCHIDA K, et al. Role of interferon-gamma in inflammatory responses in murine respiratory infection with *Legionella pneumophila* [J]. J Med Microbiol, 2002, 51(3): 225-30.
[57] PANDA S K, COLONNA M. Innate lymphoid cells in mucosal immunity [J]. Front Immunol, 2019, 10: 861.
[58] SALUZZO S, GORKI A D, RANA B M J, et al. First-breath-induced type 2 pathways shape the lung immune environment [J]. Cell Rep, 2017, 18(8): 1893-905.
[59] MUNOZ N, VAN MAELE L, MARQUES J M, et al. Mucosal administration of flagellin protects mice from S*treptococcus pneumoniae* lung infection [J]. Infect Immun, 2010, 78(10): 4226-33.
[60] XIONG H, KEITH J W, SAMILO D W, et al. Innate lymphocyte/Ly6C(hi) monocyte crosstalk promotes klebsiella pneumoniae clearance [J]. Cell, 2016, 165(3): 679-89.
[61] ARDAIN A, PORTERFIELD J Z, KLOVERPRIS H N, et al. Type 3 ILCs in lung disease [J]. Front Immunol, 2019, 10: 92.
[62] MARTINS S, DE PERROT M, IMAI Y, et al. Transbronchial administration of adenoviral-mediated interleukin-10 gene to the donor improves function in a pig lung transplant model [J]. Gene Ther, 2004, 11(24): 1786-96.
[63] SCHAERLI P, MOSER B. Chemokines: control of primary and memory T-cell traffic [J]. Immunol Res, 2005, 31(1): 57-74.
[64] ALLEN S J, CROWN S E, HANDEL T M. Chemokine: receptor structure, interactions, and antagonism [J]. Annu Rev Immunol, 2007, 25: 787-820.
[65] WANG Y, ZHANG Y, YANG X, et al. Chemokine-like factor 1 is a functional ligand for CC chemokine receptor 4 (CCR4) [J]. Life Sci, 2006, 78(6): 614-21.
[66] WANG Y, ZHANG Y, HAN W, et al. Two C-terminal peptides of human CKLF1 interact with the chemokine receptor CCR4 [J].

Int J Biochem Cell Biol, 2008, 40(5): 909-19.

[67] ZHANG Y, TIAN L, ZHENG Y, et al. C-terminal peptides of chemokine-like factor 1 signal through chemokine receptor CCR4 to cross-desensitize the CXCR4 [J]. Biochem Biophys Res Commun, 2011, 409(2): 356-61.

[68] ZHENG Y, GUO C, ZHANG Y, et al. Alleviation of murine allergic rhinitis by C19, a C-terminal peptide of chemokine-like factor 1 (CKLF1) [J]. Int Immunopharmacol, 2011, 11(12): 2188-93.

[69] HESSVIK N P, LLORENTE A. Current knowledge on exosome biogenesis and release [J]. Cell Mol Life Sci, 2018, 75(2): 193-208.

[70] MAAS S L N, BREAKEFIELD X O, WEAVER A M. Extracellular vesicles: unique intercellular delivery vehicles [J]. Trends Cell Biol, 2017, 27(3): 172-88.

[71] MIGNOT G, ROUX S, THERY C, et al. Prospects for exosomes in immunotherapy of cancer [J]. J Cell Mol Med, 2006, 10(2): 376-88.

[72] THERY C, OSTROWSKI M, SEGURA E. Membrane vesicles as conveyors of immune responses [J]. Nat Rev Immunol, 2009, 9(8): 581-93.

[73] WUBBOLTS R, LECKIE R S, VEENHUIZEN P T, et al. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation [J]. J Biol Chem, 2003, 278(13): 10963-72.

[74] BARD M P, HEGMANS J P, HEMMES A, et al. Proteomic analysis of exosomes isolated from human malignant pleural effusions [J]. Am J Respir Cell Mol Biol, 2004, 31(1): 114-21.

[75] CHAPUT N, TAIEB J, ANDRE F, et al. The potential of exosomes in immunotherapy [J]. Expert Opin Biol Ther, 2005, 5(6): 737-47.

[76] BOBRIE A, COLOMBO M, RAPOSO G, et al. Exosome secretion: molecular mechanisms and roles in immune responses [J]. Traffic, 2011, 12(12): 1659-68.

[77] SCHOREY J S, BHATNAGAR S. Exosome function: from tumor immunology to pathogen biology [J]. Traffic, 2008, 9(6): 871-81.

[78] JEPPESEN D K, FENIX A M, FRANKLIN J L, et al. Reassessment of exosome composition [J]. Cell, 2019, 177(2): 428-45,e418.

[79] HUANG X, YUAN T, TSCHANNEN M, et al. Characterization of human plasma-derived exosomal RNAs by deep sequencing [J]. BMC Genom, 2013, 14: 319.

[80] VINCENT-SCHNEIDER H, STUMPTNER-CUVELETTE P, LANKAR D, et al. Exosomes bearing HLA-DR1 molecules need dendritic cells to efficiently stimulate specific T cells [J]. Int Immunol, 2002, 14(7): 713-22.

[81] CHENG Y, SCHOREY J S. Exosomes carrying mycobacterial antigens can protect mice against *Mycobacterium tuberculosis* infection [J]. Eur J Immunol, 2013, 43(12): 3279-90.

[82] GIRI P K, SCHOREY J S. Exosomes derived from M. Bovis BCG infected macrophages activate antigen-specific $CD4^+$ and $CD8^+$ T cells *in vitro* and *in vivo* [J]. PLoS One, 2008, 3(6): e2461.

[83] WANG J, DENG Z, WANG Z, et al. MicroRNA-155 in exosomes secreted from helicobacter pylori infection macrophages immunomodulates inflammatory response [J]. Am J Transl Res, 2016, 8(9): 3700-9.

[84] CHENG Y, SCHOREY J S. Extracellular vesicles deliver Mycobacterium RNA to promote host immunity and bacterial killing [J]. EMBO Rep, 2019, 20(3): e46613.

[85] NANDAKUMAR R, TSCHISMAROV R, MEISSNER F, et al. Intracellular bacteria engage a STING-TBK1-MVB12b pathway to enable paracrine cGAS-STING signalling [J]. Nat Microbiol, 2019, 4(4): 701-13.

[86] MONTECALVO A, LARREGINA A T, SHUFESKY W J, et al. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes [J]. Blood, 2012, 119(3): 756-66.

[87] ALTAN-BONNET N. Extracellular vesicles are the Trojan horses of viral infection [J]. Curr Opin Microbiol, 2016, 32: 77-81.

[88] LI J, LIU K, LIU Y, et al. Exosomes mediate the cell-to-cell transmission of IFN-alpha-induced antiviral activity [J]. Nat Immunol, 2013, 14(8): 793-803.

[89] KHATUA A K, TAYLOR H E, HILDRETH J E, et al. Exosomes packaging APOBEC3G confer human immunodeficiency virus resistance to recipient cells [J]. J Virol, 2009, 83(2): 512-21.

[90] MATTHAY M A, WARE L B, ZIMMERMAN G A. The acute respiratory distress syndrome [J]. J Clin Invest, 2012, 122(8): 2731-40.

[91] LIU F, PENG W, CHEN J, et al. Exosomes derived from alveolar epithelial cells promote alveolar macrophage activation mediated by miR-92a-3p in sepsis-induced acute lung injury [J]. Front Cell Infect Microbiol, 2021, 11: 646546.

[92] YE C, LI H, BAO M, et al. Alveolar macrophage-derived exosomes modulate severity and outcome of acute lung injury [J]. Aging, 2020, 12(7): 6120-8.

[93] BOURDONNAY E, ZASLONA Z, PENKE L R, et al. Transcellular delivery of vesicular SOCS proteins from macrophages to epithelial cells blunts inflammatory signaling [J]. J Exp Med, 2015, 212(5): 729-42.

[94] LEE H, ZHANG D, LASKIN D L, et al. Functional evidence of pulmonary extracellular vesicles in infectious and noninfectious lung inflammation [J]. J Immunol, 2018, 201(5): 1500-9.

[95] ZHANG D, LEE H, WANG X, et al. A potential role of microvesicle-containing miR-223/142 in lung inflammation [J]. Thorax, 2019, 74(9): 865-74.

[96] SINGH P P, LI L, SCHOREY J S. Exosomal RNA from Mycobacterium tuberculosis-infected cells is functional in recipient macrophages [J]. Traffic, 2015, 16(6): 555-71.

[97] GENSCHMER K R, RUSSELL D W, LAL C, et al. Activated PMN exosomes: pathogenic entities causing matrix destruction and disease in the lung [J]. Cell, 2019, 176(1/2): 113-26,e115.

[98] CHENG L, SHARPLES R A, SCICLUNA B J, et al. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood [J]. J Extracell Vesicles, 2014, doi: 10.3402/jev.v3.23743. eCollection 2014.

[99] RABINOWITS G, GERCEL-TAYLOR C, DAY J M, et al. Exosomal microRNA: a diagnostic marker for lung cancer [J]. Clin Lung Cancer, 2009, 10(1): 42-6.

[100] SANDFELD-PAULSEN B, JAKOBSEN K R, BAEK R, et al. Exosomal proteins as diagnostic biomarkers in lung cancer [J]. J Thorac Oncol, 2016, 11(10): 1701-10.

[101] AKBARI A, REZAIE J. Potential therapeutic application of mes-

enchymal stem cell-derived exosomes in SARS-CoV-2 pneumonia [J]. Stem Cell Res Ther, 2020, 11(1): 356.

[102] SENGUPTA V, SENGUPTA S, LAZO A, et al. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19 [J]. Stem Cells Dev, 2020, 29(12): 747-54.

[103] FERGUSON S W, NGUYEN J. Exosomes as therapeutics: the implications of molecular composition and exosomal heterogeneity [J]. J Control Release, 2016, 228: 179-90.

[104] WALKER S, BUSATTO S, PHAM A, et al. Extracellular vesicle-based drug delivery systems for cancer treatment [J]. Theranostics, 2019, 9(26): 8001-17.

[105] ZHANG D, LEE H, ZHU Z, et al. Enrichment of selective miRNAs in exosomes and delivery of exosomal miRNAs *in vitro* and *in vivo* [J]. Am J Physiol Lung Cell Mol Physiol, 2017, 312(1): L110-21.