外泌体影响急性胰腺炎多脏器损伤的研究进展

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摘要 急性胰腺炎(acute pancreatitis, AP)是临床常见的急腹症, 以胰腺为发病中心, 随着病程的发展可诱导机体产生全身性炎症反应, 累及肺、肠、肝脏及肾脏等身体多个器官。外泌体是由细胞产生的携带多种活性物质的细胞外囊泡, 可充当细胞间信息交流的介质。近年来研究发现临床AP患者血浆外泌体存在显著特异性改变, 相关基础研究进一步揭示了外泌体在AP多脏器损伤中的关键作用。该文就外泌体的生物发生过程及其在AP多脏器损伤中的作用机制进行综述, 以进一步明确其影响AP多脏器损伤的具体机制, 为AP临床治疗提供新的方向。

关键词 外泌体; 急性胰腺炎; 多脏器损伤; 发病机制

Research Progress on Effects of Exosomes on Multiple Organ Injury in Acute Pancreatitis

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Abstract AP (acute pancreatitis) is a clinically common acute abdominal disorder centered on the pancreas that over the course of the disease can induce systemic inflammation that affects multiple organs of the body, including the lungs, intestines, liver, and kidneys. Exosomes are extracellular vesicles produced by cells that carry a variety of active substances and can act as mediators for information exchange between cells. Recent studies have found significant specific changes in plasma exosomes in patients with clinical AP, and relevant basic studies have further revealed the key role of exosomes in AP multiple organ injury. In this paper, the biogenetic process of exosomes and the mechanism of action in AP multiple organ injury were reviewed, so as to further clarify the specific mechanism of exosomes affecting AP multiple organ injury, and provided a new direction for clinical treatment of AP.

Keywords exosome; acute pancreatitis; multiple organ injury; pathogenesis

外泌体作为细胞外囊泡(extracellular vesicles, EVs)的一种, 可由几乎所有类型细胞内源性分泌; 外泌体在生物体内分布广泛, 在血液、唾液、母乳、脑脊液、胆汁、腹水、支气管肺泡灌洗液等各种体液中均有发现[1-2]。最初的研究认为, 外泌体仅是在网织红细胞成熟过程中去除转铁蛋白受体的排泄囊

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泡, 是一种细胞废物处理机制[3-4], 而大量研究发现外泌体是一种新的调节细胞间通讯的重要途径, 对疾病的发生发展有关键影响[5-6]。外泌体在机体免疫调控、抗原提呈、细胞生命活动调节等方面具有重要作用[7]。

急性胰腺炎(acute pancreatitis, AP)是一种临床常见的急腹症, 其发生发展以胰腺为中心, 病程累及多个器官, 肺部、肠道、肝脏、肾脏等作为AP关联器官也常受到不同程度的损伤。据统计全球每年AP发病率约为34/10万, 死亡率约为1.16/10万, 且发病率呈上升趋势[8-9]。研究表明, 在AP胰腺组织受损、炎症反应和多脏器损伤的过程中血浆外泌体浓度及表型发生显著变化并参与了AP进程[10]。在AP发病过程中, 胰腺腺泡细胞受到胰酶及多种炎症介质刺激释放外泌体进入循环系统参与炎症反应, 受损的胰腺腺泡细胞释放的外泌体可到达肝脏、肾脏、脾、肠道、肺等器官并激活巨噬细胞, 促使被激活的巨噬细胞分泌大量促炎细胞因子进入血液循环, 从而产生全身炎症反应(systemic inflammatory response syndrome, SIRS)甚至多器官功能障碍综合征[11]。本文对外泌体的生物发生过程及其在AP相关多器官损伤中的作用机制进行综述, 以期推动急性胰腺炎及相关多脏器损伤的临床和基础研究取得进一步突破。

1 外泌体: 生物发生、内容物和功能

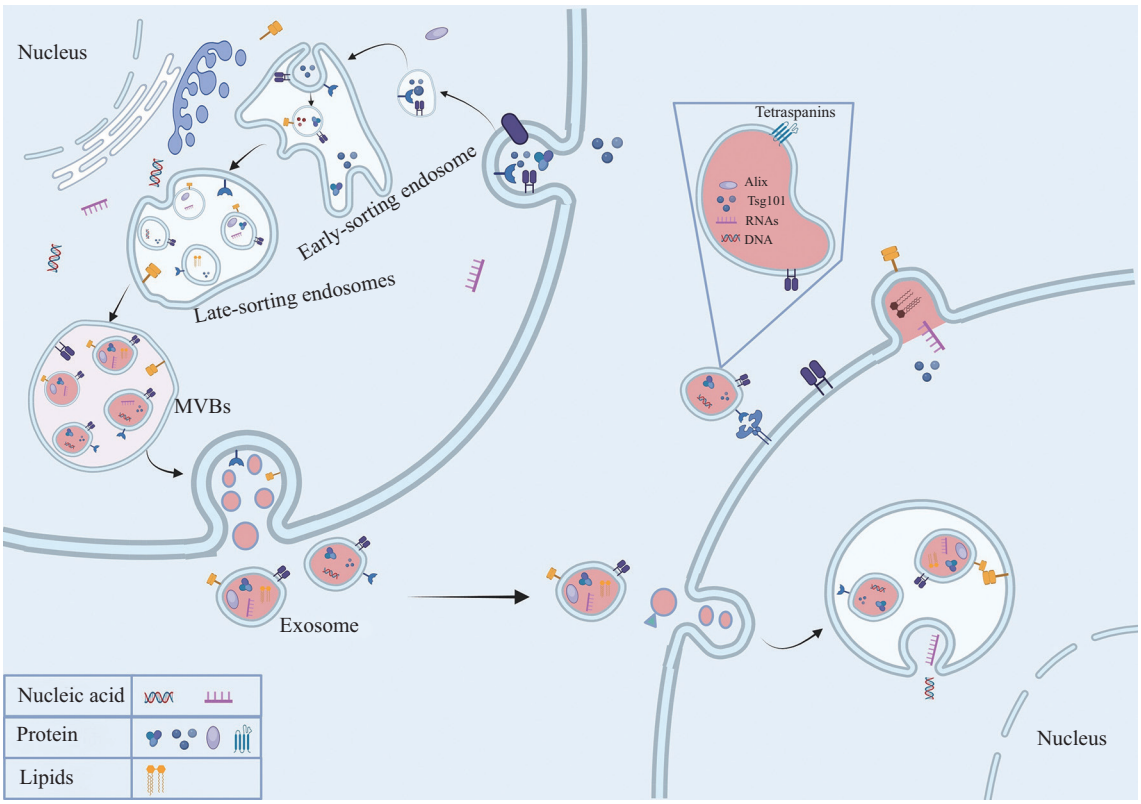
近年来外泌体相关研究规模迅速扩大, 其生物发生、内容物构成以及发挥功能的途径已逐步被发现(图1)[12-13]。外泌体是纳米级的, 脂质双层膜封闭的胞外囊泡, 透射电镜显示外泌体具有经典的“杯”状或“盘”状结构, 平均粒径约100纳米, 主要成分为核酸、蛋白质、脂质等[14]。外泌体来源于内吞系统中多囊泡体(multivesicular bodies, MVBs)内包含的腔内囊泡(intralumenal vesicles, ILVs)[15], 其起源、合成和分泌经历以下过程: 母细胞的细胞膜通过胞吞或“向内出芽”形成早期内涵体, 早期内涵体在细胞内部逐渐成熟为晚期内涵体, 并最终生成MVBs。外泌体的雏形以ILVs的形式存在于MVBs内部, 随后MVBs再次与细胞质膜融合, 以外吐的方式将ILVs排出细胞成为外泌体[16]。

外泌体已被广泛证明携带mRNA和非编码RNA(non-coding RNA, ncRNA), 如微小RNA(miRNA)或长链非编码RNA(lncRNA), 它们可以在组织细胞间转移, 影响翻译进程和受体细胞中蛋白的表达[17]。miRNA是一种内源性非编码单链小RNA分子, 长度约22个核苷酸, 具有茎环结构; miRNA在生物体中高度保守, 可以与Argonaute蛋白形成RNA诱导的沉默复合物以诱导靶基因mRNA降解或抑制其翻译, 在转录后水平上调节基因的表达[18-19]。众多研究表明细胞外存在大量的miRNA, 而miRNA是外泌体递送RNA的主要形式, 其可以被包裹在外泌体中以规避降解进而介导细胞间通讯及参与基因调控[20]。lncRNA是ncRNA的一种, 长度超过200个核苷酸, 其合成方式类似于mRNA, lncRNA初始被认为是无意义的转录噪声[21], 但越来越多的证据表明lncRNA可能在表观遗传与基因调控中起重要作用[22-23]。外泌体包含保守蛋白, 如膜联蛋白、筏蛋白, 四跨膜蛋白(CD9、CD63等)、热休克蛋白(HSP70、HSP90等)、凋亡转接基因2互作蛋白X(apoptosis linked gene-2-interacting protein X, Alix)、MHC相关蛋白、肿瘤易感基因101(tumor susceptibility gene 101, Tsg101)以及其他类型的特异性蛋白, 它们反映了外泌体的来源[24]。作为外泌体最常见的装载物, 这些特异性蛋白主要存在于膜表面和腔内, 理化性质较稳定, 不易受到细胞外环境的影响, 并且可能在疾病进展中发挥关键作用[25]。

外泌体作为胞间通讯介质, 其活动具有不可预测性并易受外界环境变化的影响。研究发现外泌体既存在目的明确的定向迁徙, 如受体介导的特异性结合或肿瘤细胞的“归巢”[26-27]; 也存在随机性运动, 它们可以被循环系统或网状内皮系统非特异性吞噬, 也可能因受体细胞特定微环境及细胞自身状态的变化而受到影响[28]。外泌体被靶细胞接收进而发挥其生物学功能的途径目前主要包括以下几种: 外泌体膜表面配体可以与靶细胞膜表面的受体结合, 激活受体介导的信号通路; 受体细胞也可将外泌体以胞吞形式摄入胞内, 外泌体内容物在胞内释放后执行其信使功能或参与新的MVBs或细胞器的合成; 外泌体膜还可以直接与受体细胞膜融合, 将内容物释放入细胞质中[5,29]。

2 外泌体在AP多脏器损伤发病机制中的作用

AP的发生发展常伴随着肺脏、肝脏、肠道和



Alix: 凋亡转接基因2互作蛋白X; Tsg101: 肿瘤易感基因101。

Alix: apoptosis linked gene-2-interacting protein X; Tsg101: tumor susceptibility gene 101.

图1　外泌体生物发生、内容物和功能(根据参考文献[13]修改)

Fig.1　Exosome biogenesis, content and function (modified from reference [13])

肾脏等器官的损伤，而免疫细胞和实质细胞间的信号转导是促进AP多脏器损伤和炎症级联反应的关键，在此过程中外泌体具有至关重要的作用。既往研究表明，外泌体可通过其包含的的特异性ncRNA和蛋白质影响AP及相关多脏器损伤[10-11]。

2.1　外泌体在AP胰腺损伤中的作用

外泌体组分不仅是液体活检的高价值信息分子，也是外泌体发挥细胞调控功能的重要效应分子(表1)。ZHAO等[10]在进行AP与巨噬细胞活化的机制研究时发现，使用牛磺胆酸钠(taurocholic acid sodium salt, TLCS)刺激胰腺腺泡细胞(AR42J细胞)复制AP模型后，活化的AR42J细胞可分泌携带有差异表达miRNA的外泌体并显著增强巨噬细胞中核转录因子κB(nuclear factor kappa-B, NF-κB)的活化，外泌体miRNA微阵列鉴定及KEGG通路富集分析表明MAPK信号通路与巨噬细胞和NF-κB的活化密切相关，miR-128-3p、miR-423-5p及miR-665等差异表达的miRNA作为介质可能通过调节TRAF6/IKK/NF-κB通路的各个步骤参与AP进程。AP的发生常伴随大量炎症因子释放和细胞死亡，miR-24-3p参与了这一过程，AR42J细胞在应激状态下可产生高表达miR-24-3p的外泌体抑制March3的表达，进而促进腹膜巨噬细胞M1极化和焦亡并加重腺泡细胞损伤，而miR-24-3p抑制剂抵消了胰腺外泌体对腹膜巨噬细胞M1极化和焦亡的影响[30]。TANG等[31]发现在AP的初始阶段，腺泡细胞来源的外泌体miR-183-5p可抑制叉头盒蛋白O1(forkhead box protein O1, FoxO1)表达导致M1巨噬细胞极化并抑制其吞噬作用，且miR-183-5p在巨噬细胞中过表达能诱导腺泡细胞损伤和胰蛋白酶活化，从而进一步加重AP。miR-21-5p在AP酶噬进程中发挥关键作用，WANG等[32]使用TLCS刺激AR42J细胞活化后发现miR-21-5p表达水平显著升高并抑制了由Trim33(一种介导胰蛋白酶泛素化的关键E3连接酶)介导的酶噬反应，敲低miR-21-5p的表达或使用其竞争性抑制剂能有效改善AP炎症反应，具体而言miR-21-5p可能通过抑制Trim33的表达调节VMP1介导的酶噬过程进而加剧AP炎症反应。

转移相关肺腺癌转录本1(metastasis associated

lung adenocarcinoma transcript 1, MALAT1)作为外泌体中最早发现的lncRNA之一, 与炎症性疾病的发生发展密切相关[33]。临床研究发现AP患者血浆中提取的EVs中MALAT1水平较健康人群显著升高, LIU等[34]发现雨蛙肽(cerulein)刺激小鼠胰腺腺泡细胞可产生携带MALAT1的外泌体并诱导炎症反应, 提示在临床AP患者血浆中观察到携带了MALAT1的外泌体可能来自受损的腺泡细胞, 研究人员发现MALAT1可能通过miR-181a-5p/TLR4/NF-κB/HMGB1轴促进巨噬细胞M1极化并加剧AP损伤。GU等[35]通过研究发现AP腺泡细胞的早期炎症反应可能存在“MALAT1-miR-194-yes相关蛋白1(Yes associated protein 1, YAP1)”的动态调控回路, 在AP细胞模型中检测到MALAT1和YAP1的表达以及炎症因子水平显著升高, 而miR-194可通过靶向YAP通路来抑制AP细胞模型的炎症反应。LncRNA牛磺酸上调基因1(taurine upregulated gene 1, TUG1)由7 598个核苷酸组成, 在AP发病过程中TUG1可能参与了AP引起的免疫应答失调; WEN等[36]在AR42J细胞复制的AP体外模型中观察到TUG1和外泌体TUG1的表达水平显著上调, 过表达的外泌体TUG1可能通过介导Treg细胞分化抑制机体免疫反应从而促进胰腺腺泡细胞的凋亡和炎症反应。

S100A8和S100A9均属于S100蛋白家族, 由低分子量的钙结合蛋白组成, 其参与AP病程中多种细胞信号转导过程[37-38]。CARRASCAL等[39-40]发现从重症急性胰腺炎(severe acute pancreatitis, SAP)患者血浆中分离出的外泌体特异性S100A8和S100A9水平显著上升并促进了NF-κB信号通路激活和巨噬细胞中炎症介质的释放; 另一项研究也发现酒精性AP患者的血浆S100A8显著上调, 这些发现表明外泌体特异性S100A8和S100A9与AP期间SIRS的强度相关。组蛋白是真核细胞中的基本蛋白质, 与DNA结合形成染色质并调节基因表达; 细胞在应激状态下可能将组蛋白从细胞核转移到细胞外空间, 引发机体免疫反应并加重炎症[41]。组蛋白在AP起始过程中发挥关键作用。大量关于EVs的蛋白质组学鉴定表明细胞可能分泌含有组蛋白的外泌体, 在外泌体中也发现了N-端裂解的组蛋白H3和H2A[42-43]。GUO等[44]通过研究发现细胞外组蛋白可以通过激活质膜TLR9诱导AR42J细胞出现剂量依赖性钙振荡, 而TLR9是胰蛋白酶活化和AP期间炎症信号通路激活的关键受体, 这一结果表明细胞外组蛋白可能是诱导AP损伤的重要介质之一。

2.2 外泌体在AP肺损伤中的作用

肺功能障碍是AP并发症中最早和最重要的。在SAP早期引发的所有死亡中, 60%是由肺功能障碍造成的[16]。急性肺损伤(acute lung injury, ALI)是肺功能障碍的主要形式之一, 其特征是肺微血管内皮细胞(pulmonary microvascular endothelial cells, PMVECs)和肺泡上皮细胞(alveolar epithelial cells, AECs)损伤所致的弥漫性间质和肺泡水肿[45-46]。由AP诱导而差异表达的外泌体ncRNA和蛋白质等是导致肺部血液屏障破坏和肺组织损伤的关键因素之一(表2)。

BONJOCH等[47]通过研究发现外泌体参与急性胰腺炎相关急性肺损伤(acute pancreatitis with acute lung injury, AP-ALI), 在AP过程中循环外泌体水平

表1 外泌体参与AP胰腺损伤

Table 1 The involvement of exosomes in AP pancreatic injury

种类 Types	名称 Name	在AP中的表达和作用 Expression and function in AP	目标/通路 Target/path	参考文献 References
miRNA	miR-128-3p, miR-15b-5p, miR-423-5p, miR-679, miR-665, miR-151-5p, miR-761	Down, aggravated	TRAF6-TAB2-TAK1-NIK/IKK-NF-κB	[10]
	miR-24-3p	Up, aggravated	MARCH3/NLRP3	[30]
	miR-183-5p	Up, aggravated	FoxO1	[31]
	miR-21-5p	Up, aggravated	Trim33/VMP1	[32]
lncRNA	MALAT1	Up, aggravated	miR-181a-5p/TLR4/NF-κB/HMGB1, miR-194/YAP1	[34-35]
	TUG1	Up, aggravated	Treg	[36]
Protein	S100A8, S100A9	Up, aggravated	NF-κB	[39-40]
	Histones	Up, aggravated	TLR9	[44]

显著升高并表现出促炎特性，使用PKH26染色外泌体进行追踪后发现外泌体可以穿透肺泡内皮屏障被巨噬细胞摄入进而激活肺泡巨噬细胞(alveolar macrophage, AM)转变为炎症表型，引起AP-ALI，提示循环外泌体可能在介导全身炎症级联反应中起类似于炎症因子的作用。WU等[48]也在AP-ALI动物模型中发现大量的外泌体被释放到血液循环中激活NLRP3炎症小体并诱导AM焦亡。miR-216a作为AP发生的潜在标志物此前已经进行了广泛的研究[49]。ZHU等[50]通过研究发现AP-ALI患者的血浆外泌体miR-216a表达水平显著升高，且体内研究发现miR-216a在除胰腺以外的所有器官中均未被检测到，提示外泌体miR-216a可能由胰腺特异性产生，miR-216a过表达可通过改变紧密连接(tight junction, TJ)蛋白的表达来增加肺部微血管内皮细胞通透性，这加剧了AP-ALI的严重程度；朱惠云[51]的研究也确认了外泌体miR-216a对AP-ALI的破坏作用。miR-127在肺部疾病中起关键作用，SHI等[52]在TLCS诱导的AP-ALI模型中发现肺组织外泌体miR-127水平特异性升高，并且与胰腺和肺组织病理学损伤程度呈正相关，miR-127与促炎细胞因子(IL-1β、IL-6和TNF-α)表达量均增加。YING等[53]发现miR-127可能通过Bcl6/Dusp1/JNK轴级联调控巨噬细胞M1极化并抑制其M2表型，表明miR-127可通过激活炎症通路促进肺部损伤，这些研究表明肺部的miR-127水平可能反映了胰腺和肺组织损伤，并在炎症信号转导和肺部病理学中发挥潜在作用。但值得注意的是另一项研究发现miR-127在急性肺损伤模型中表达水平显著降低，miR-127可能通过靶向CD64并影响TLR和FcγR间的串扰通路发挥治疗作用，对临床AP并发肺部损伤的患者血清检测亦呈现出相同的趋势，上述结果表明miR-127在AP诱导的肺损伤中可能表现出双重调节作用[54]。

冷诱导型RNA结合蛋白(cold-inducible RNA-binding protein, CIRP)是存在于哺乳动物体内的冷休克蛋白，在低温或缺氧时应激表达[55]。研究表明，从细胞中释放的CIRP在介导AP相关炎症级联反应中起到关键作用，MURAO等[56]发现CIRP可能通过外泌体在细胞外持续存在。LIU等[57]在AP模型中发现血清CIRP水平显著升高，高表达的CIRP可以直接损害胰腺腺泡细胞，并通过TLR4促进线粒体功能障碍和活性氧积累，激活NLRP3/Caspase-1焦亡信号通路促进AP进展，抑制CIRP的表达可以有效改善这种现象。XU等[58]也发现SAP-ALI模型大鼠血清和肺部的CIRP高水平表达，CIRP促进SAP-ALI可能是通过激活NLRP3/IL-1β/CXCL1通路实现的，使用C23(CIRP的拮抗剂)可显著抑制SAP-ALI的进展。可溶性异柠檬酸脱氢酶1(isocitrate dehydrogenase 1, IDH1)在SAP炎症反应中发挥重要作用，HU等[11]发现在SAP-ALI大鼠模型中血浆外泌体特异性蛋白IDH1表达水平显著升高，AM被激活并表现为促炎性，提示SAP来源的外泌体可以诱导巨噬细胞M1型极化，IDH1可能通过抑制IκB磷酸化激活NF-κB信号通路，并通过调节组蛋白甲基化下调PPARγ的表达，进而加重SAP-ALI。ITGAM和ITGB2可异二聚化形成整合素αmβ2，参与细胞迁移和黏附。HU等[59]观察到SAP模型来源的EVs中ITGAM或ITGB2高表达且可能通过破坏PMVECs屏障，促使炎症因子和活化的免疫细胞进入肺泡放大炎症反应，并促进SAP-ALI发展。

2.3 外泌体在AP肠损伤中的作用

AP常导致肠道功能障碍，肠屏障损伤和肠道通透性增加在AP患者和各种AP模型中普遍存在[60]。由于肠道损伤，后续的放大效应会进一步加剧AP引起的全身炎症反应[61]。在此期间，外泌体及其特异性miRNA和蛋白质发挥了重要作用(表3)。

YANG等[62]发现SAP模型中循环外泌体水平显著上升并激活了NLRP3炎症小体介导的肠上皮细胞

表2 外泌体参与AP-ALI

Table 2 Participation of exosomes in AP-ALI

种类 Types	名称 Name	在AP中的表达和作用 Expression and function in AP	目标/通路 Target/pathway	参考文献 References
miRNA	miR-216a	Up, aggravated	PMVECs	[50-51]
	miR-127	Up, aggravated, down, suppressive	IL-1β, TNF-α, IL-6, Bcl6/Dusp1/JNK, CD64	[52-54]
Protein	CIRP	Up, aggravated	TLR4, NLRP3/IL-1β/CXCL1	[57-58]
	IDH1	Up, aggravated	NF-κB, PPARγ	[11]
	ITGAM, ITGB2	Up, aggravated	PMVECs	[59]

(intestinal epithelial cells, IECs)焦亡, 而GW4869(外泌体合成/释放抑制剂)显著抑制了IECs的炎症反应, 表明抑制循环外泌体释放可有效改善NLRP3炎症小体介导的IECs焦亡并减轻SAP诱导的肠屏障损伤。另一项研究发现来源于SAP模型的外泌体可显著诱导IECs焦亡和肠屏障丢失, 外泌体中高表达的miR-155-5p可能发挥了关键作用, miR-155-5p可靶向SOCS1/NLRP3通路激活炎症小体介导的细胞焦亡, 导致肠屏障损伤[63]。TIAN等[64]也在脂多糖(lipopolysaccharide, LPS)联合雨蛙素(LPS/cerulein)诱导的急性胰腺炎伴急性肠损伤(acute pancreatitis with acute intestinal injury, AP-AII)模型中发现大量炎症因子释放并诱导miR-155在IECs中显著过表达, 高水平的miR-155可抑制肠屏障关键蛋白的表达并加剧AP-AII, miR-155的破坏作用可能是通过激活TNF-α/miR-155/RhoA信号通路实现的。miR-21也介导了AP-AII, ZHANG等[65]发现在炎症诱导的肠屏障损伤中, 外泌体特异性miR-21可通过PTEN/PI3K/AKT信号通路调节IECs通透性, 促进炎症因子释放进而导致肠道功能紊乱。PARK等[66]提出IECs在应激条件(氧化应激、代谢障碍或缺血再灌注等)下释放的外泌体可能介导了SIRS的产生, 在生理条件下, IECs的外泌体主要参与协调肠道免疫调节并维持免疫稳态, 当AP发生时, 受损的IECs可能释放miR-122a和miR-29a等miRNA, 高表达的miR-122a和miR-29a可通过下调肠上皮TJ蛋白的表达来增加肠道通透性并加剧肠屏障损伤。

高迁移率族蛋白B1(highmobility group box 1, HMGB1)是一种重要的损伤相关分子模式, 有学者发现其可能通过外泌体途径释放[67]。CHEN等[68]在AP并发肠黏膜屏障功能障碍的机制研究中发现LPS/cerulein诱导的AP模型HMGB1表达水平显著增加, 并促进了炎症因子释放, HMGB1可能通过激活TLR4/9/NF-κB信号通路介导AP相关肠屏障功能障碍。LIU等[69]也在炎症诱导的Caco-2细胞囊泡中发现HMGB1, 表明该蛋白至少部分来自肠道细胞分泌的外泌体, 并且此过程可能通过自分泌反馈回路加剧炎症诱导的肠屏障损伤。

2.4 外泌体在AP其他器官损伤中的作用

AP并发肝损伤发病率较高。肝细胞与腺泡细胞类似, 肝细胞损伤和随后的外泌体释放会促进AP发展, 参与炎症调节的外泌体相关miRNA在疾病进展期间扮演了关键角色(表4)。BALA等[70]通过研究发现肝脏受损时可分泌异常表达的外泌体, 外泌体中的miRNA在不同类型的肝损伤中表现出多种富集形式, 其中miR-122可作为肝损伤的潜在标志物, 其表达与丙氨酸氨基转移酶(Alanine aminotransferase, ALT)相关, miR-155可作为炎症标志物, 通过NF-κB信号通路调节肝细胞炎症反应。miR-155是一种由非编码B细胞整合簇编码的miRNA, 在机体的炎症反应和免疫应答中具有至关重要的调节作用[71]。JIMÉNEZ-ALESANCO等[72]在进行了一系列研究后发现AP期间激活全身炎症反应的血浆外泌体来源可能是肝脏而不是胰腺, 研究人员通过检测AP模型大鼠血浆和腹水外泌体在miRNA含量、分布以及蛋白质组学的差异时发现: 血浆外泌体包含高表达的miR-155和低表达的miR-122和miR-21, 而这些miRNA在腹水的外泌体中表达与对照组无差异; 此外, 蛋白质组学分析表明, 血浆外泌体的蛋白质主要来自肝脏, 仅检测到两种胰腺特异性蛋白; 在体外研究中, 血浆外泌体表现出远高于腹水中外泌体的促炎活性, 上述结果都表明在AP期间血浆外泌体更可能来源于肝脏。HU等[73]发现AP患者的外泌体特异性miR-192-5p水平显著下调, 且AP合并肝损伤患者的循环miR-192-5p水平显著低于健康对照组, 体外研究表明miR-192-5p可能通过促进胰腺腺泡细胞凋亡和抑制炎症

表3 外泌体参与AP-AII

Table 3 Participation of exosomes in AP-AII

种类 Types	名称 Name	在AP中的表达和作用 Expression and function in AP	目标/通路 Target/pathway	参考文献 References
miRNA	miR-155-5p	Up, aggravated	SOCS1/NLRP3	[63]
	miR-155	Up, aggravated	TNF-α/RhoA	[64]
	miR-21	Up, aggravated	PTEN/PI3K/AKT	[65]
	miR-122a, miR-29a	Up, aggravated	TJ	[66]
Protein	HMGB1	Up, aggravated	TLR4/9/NF-κB	[68-69]

表4 外泌体在AP其他外器官损伤中的作用

Table 4 The role of exosomes in the injury of other external organs of AP

种类 Types	名称 Name	在AP中的表达和作用 Expression and function in AP	目标/通路 Target/pathway	参考文献 References
miRNA	miR-122	Up	ALT	[70]
	miR-155	Up, aggravated	NF-κB	[70,72]
	miR-192-5p	Down, suppressive	PTEN/PI3K/AKT	[73]
mRNA	CCL2	Up, aggravated	Macrophage	[74]

发挥对AP肝损伤的保护作用。

此外, 急性肾损伤也是AP常见的并发症之一。LÜ等[74]通过研究发现在急性肾损伤发生时, 肾脏和尿液中含有炎症细胞因子mRNA的外泌体显著增加, 趋化因子CCL2的mRNA在外泌体中高水平表达并可被巨噬细胞内化导致肾周炎性细胞浸润进而加剧肾损伤, 抑制CCL2的表达可有效改善上述情况。

3 总结与展望

随着近年来外泌体研究的深入和扩展, 其生物发生过程、内容物和功能已逐步被发现。本文总结了外泌体在AP及相关多脏器损伤中发挥的作用, 外泌体中的核酸与蛋白质可通过激活炎症相关信号通路、影响巨噬细胞极化、干预酶噬进程、介导机体免疫反应和诱导腺泡细胞钙振荡等途径参与AP进程, 同时外泌体作为机体组织和细胞间信息传递的媒介, 参与AP多脏器损伤。在AP-ALI过程中, 外泌体可通过激活肺泡巨噬细胞活化、破坏肺部血液屏障、影响线粒体功能并诱导细胞焦亡及参与炎症反应介导AP-ALI, 同时也可通过调节机体免疫反应发挥治疗作用, 表现出双重调节特性。外泌体还能通过激活NLRP3炎症小体介导肠道IECs焦亡、抑制肠屏障关键蛋白的表达增加肠屏障通透性以及加剧全身炎症反应影响AP-AII。在AP并发肝损伤与肾脏疾病中, 外泌体主要通过调节机体炎症反应发挥治疗作用或破坏作用, 目前有关AP并发肝、肾损伤的机制研究相对较少, 后续还需进一步探索。

参考文献 (References)

[1] ASEA A, JEAN-PIERRE C, KAUR P, et al. Heat shock protein-containing exosomes in mid-trimester amniotic fluids [J]. J Reprod Immunol, 2008, 79: 12-7.

[2] WITWER K W, BUZAS E I, BEMIS L T, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research [J]. J Extracell Vesicles, 2013, doi: 10.3402/jev.v2i0.20360.

[3] THÉRY C, ZITVOGEL L, AMIGORENA S. Exosomes: composition, biogenesis and function [J]. Nat Rev Immunol, 2002, 2(8): 569-79.

[4] WEBBER A J, JOHNSON S A. Platelet participation in blood coagulation aspects of hemostasis [J]. Am J Pathol, 1970, 60(1): 19-42.

[5] PITT J M, KROEMER G, ZITVOGEL L. Extracellular vesicles: masters of intercellular communication and potential clinical interventions [J]. J Clin Invest, 2016, 126(4): 1139-43.

[6] FAN Y, LI Z, HE Y. Exosomes in the pathogenesis, progression, and treatment of osteoarthritis [J]. Bioengineering, 2022, 9(3): 99.

[7] 梁华益, 杨复锵, 潘路娟, 等. 外泌体在急性胰腺炎发病机制、诊断及治疗中作用的研究进展[J]. 山东医药(LIANG H Y, YANG F Q, PAN L J, et al. Research progress on the role of exosomes in the pathogenesis, diagnosis and treatment of acute pancreatitis [J]. Shandong Medical Journal), 2021, 61(17): 95-7.

[8] SCHIEMER M, TREIBER M, HEEG S. Acute pancreatitis [J]. Dtsch Med Wochenschr, 2021, 146(4): 229-6.

[9] HUANG Y, BADURDEEN D S. Acute pancreatitis review [J]. Turk J Gastroenterol, 2023, 34(8): 795-801.

[10] ZHAO Y, WANG H, LU M, et al. Pancreatic acinar cells employ mirnas as mediators of intercellular communication to participate in the regulation of pancreatitis-associated macrophage activation [J]. Mediators Inflamm, 2016, 2016: 6340457.

[11] HU Q, YAO J, WU X, et al. Emodin attenuates severe acute pancreatitis-associated acute lung injury by suppressing pancreatic exosome-mediated alveolar macrophage activation [J]. Acta Pharm Sin B, 2022, 12(10): 3986-4003.

[12] SUIRE C N, HADE M D. Extracellular vesicles in type 1 diabetes: a versatile tool [J]. Bioengineering, 2022, 9(3): 105.

[13] HADE M D, SUIRE C N, SUO Z. Mesenchymal stem cell-derived exosomes: applications in regenerative medicine [J]. Cells, 2021, 10(8): 1959.

[14] KALLURI R, LEBLEU V S. The biology, function, and biomedical applications of exosomes [J]. Science, 2020, 367(6478): eaau6977.

[15] COLOMBO M, RAPOSO G, THÉRY C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles [J]. Annu Rev Cell Dev Biol, 2014, 30: 255-89.

[16] GUO X Y, XIAO F, LI J, et al. Exosomes and pancreatic diseases: status, challenges, and hopes [J]. Int J Biol Sci, 2019, 15(9): 1846-60.

[17] VALADI H, EKSTRÖM K, BOSSIOS A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanismof genetic exchange between cells [J]. Nat Cell Biol, 2007, 9: 654-9.

[18] TREIBER T, TREIBER N, MEISTER G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways [J]. Nat Rev Mol Cell Biol, 2019, 20(1): 5-20.

[19] MAIESE A, SCATENA A, COSTANTINO A, et al. Expression of micrornas in sepsis-related organ dysfunction: a systematic review [J]. Int J Mol Sci, 2022, 23(16): 9354.

[20] LIU W Z, MA Z J, KANG X W. Current status and outlook of advances in exosome isolation [J]. Anal Bioanal Chem, 2022, 414(24): 7123-41.

[21] DAHARIYA S, PADDIBHATLA I, KUMAR S, et al. Long non-coding RNA: classification, biogenesis and functions in blood cells [J]. Mol Immunol, 2019, 112: 82-92.

[22] DYKES I M, EMANUELI C. Transcriptional and post-transcriptional gene regulation by long non-coding RNA [J]. Genomics Proteomics Bioinformatics, 2017, 15(3): 177-86.

[23] ENGREITZ J M, HAINES J E, PEREZ E M, et al. Local regulation of gene expression by lncRNA promoters, transcription and splicing [J]. Nature, 2016, 539(7629): 452-5.

[24] XIN H, LI Y, CHOPP M. Exosomes/miRNAs as mediating cell-based therapy of stroke [J]. Front Cell Neurosci, 2014, 8: 377.

[25] CASERTA S, GHEZZI P. Release of redox enzymes and microRNAs in extracellular vesicles, during infection and inflammation [J]. Free Radic Biol Med, 2021, 169: 248-57.

[26] 曾凯, 许利剑. 外泌体源性miRNA在消化道肿瘤中的研究进展[J]. 医学研究生学报(ZENG K, XU L J. Research progress of exosome-derived microRNA in gastrointestinal tumors [J]. Journal of Medical Research & Combat Trauma Care), 2020, 33(1): 108-12.

[27] BECKER A, THAKUR B K, WEISS J M, et al. Extracellular vesicles in cancer: cell-to-cell mediators of metastasis [J]. Cancer Cell, 2016, 30(6): 836-48.

[28] ZOMER A, MAYNARD C, VERWEIJ F J, et al. *In vivo* imaging reveals extracellular vesicle-mediated phenocopying of metastatic behavior [J]. Cell, 2015, 161(5): 1046-57.

[29] FAROOQI A A, DESAI N N, QURESHI M Z, et al. Exosome biogenesis, bioactivities and functions as new delivery systems of natural compounds [J]. Biotechnol Adv, 2018, 36(1): 328-34.

[30] SU X J, CHEN Y, ZHANG Q C, et al. Exosomes derived from caerulein-stimulated pancreatic acinar cells mediate peritoneal macrophage M1 polarization and pyroptosis via an miR-24-3p/MARCH3/NLRP3 axis in acute pancreatitis [J]. Pancreas, 2024, doi: 10.1097/MPA.0000000000002351.

[31] TANG D S, CAO F, YAN C S, et al. Acinar cell-derived extracellular vesicle MiRNA-183-5p aggravates acute pancreatitis by promoting M1 macrophage polarization through downregulation of FoxO1 [J]. Front Immunol, 2022, 13: 869207.

[32] WANG Q, YU J, GAO W, et al. The lncRNA TCONS_00021785/miR-21-5p/Trim33 axis regulates VMP1-mediated zymophagy, reduces the activation of trypsinogen, and promotes acinar cell recovery [J]. Cell Death Discov, 2022, 8(1): 65.

[33] LAI X, ZHONG J, ZHANG A, et al. Focus on long non-coding RNA MALAT1: insights into acute and chronic lung diseases [J]. Front Genet, 2022, 13: 1003964.

[34] LIU J, NIU Z, ZHANG R, et al. MALAT1 shuttled by extracellular vesicles promotes M1 polarization of macrophages to induce acute pancreatitis via miR-181a-5p/HMGB1 axis [J]. J Cell Mol Med, 2021, 25(19): 9241-54.

[35] GU L, LIU J, XU D, et al. Reciprocal feedback loop of the MALAT1-microRNA-194-YAP1 pathway regulates progression of acute pancreatitis [J]. Med Sci Monit, 2019, 25: 6894-904.

[36] WEN X, HE B, TANG X, et al. Emodin inhibits the progression of acute pancreatitis via regulation of lncRNA TUG1 and exosomal lncRNA TUG1 [J]. Mol Med Rep, 2021, 24(5): 785.

[37] KERKHOFF C, KLEMPT M, SORG C. Novel insights into structure and function of MRP8 (S100A8) and MRP14 (S100A9) [J]. Biochim Biophys Acta, 1998, 1448(2): 200-11.

[38] WANG S, SONG R, WANG Z, et al. S100A8/A9 in inflammation [J]. Front Immunol, 2018, 9: 1298.

[39] WALDRON R T, LUGEA A, GULLA A, et al. Proteomic identification of novel plasma biomarkers and pathobiologic pathways in alcoholic acute pancreatitis [J]. Front Physiol, 2018, 9: 1215.

[40] CARRASCAL M, ARENY-BALAGUERÓ A, DE-MADARIA E, et al. Inflammatory capacity of exosomes released in the early stages of acute pancreatitis predicts the severity of the disease [J]. J Pathol, 2022, 256(1): 83-92

[41] LIGI D, GIGLIO R V, HENRY B M, et al. What is the impact of circulating histones in COVID-19: a systematic review [J]. Clin Chem Lab Med, 2022, 60(10): 1506-17.

[42] NAIR R R, MAZZA D, BRAMBILLA F, et al. LPS-challenged macrophages release microvesicles coated with histones [J]. Front Immunol, 2018, 9: 1463.

[43] PATHAN M, FONSEKA P, CHITTI S V, et al. Vesiclepedia 2019: a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles [J]. Nucleic Acids Res, 2019, 47(D1): D516-9.

[44] GUO H Y, CUI Z J. Extracellular histones activate plasma membrane toll-like receptor 9 to trigger calcium oscillations in rat pancreatic acinar tumor cell AR4-2J [J]. Cells, 2018, 8(1): 3.

[45] LIU G, ZHANG J, CHEN H, et al. Effects and mechanisms of alveolar type II epithelial cell apoptosis in severe pancreatitis-induced acute lung injury [J]. Exp Ther Med, 2014, 7(3): 565-72.

[46] VROLYK V, SINGH B. Animal models to study the role of pulmonary intravascular macrophages in spontaneous and induced acute pancreatitis [J]. Cell Tissue Res, 2020, 380(2): 207-22.

[47] BONJOCH L, CASAS V, CARRASCAL M, et al. Involvement of exosomes in lung inflammation associated with experimental acute pancreatitis [J]. J Pathol, 2016, 240: 235-45.

[48] WU X B, SUN H Y, LUO Z L, et al. Plasma-derived exosomes contribute to pancreatitis-associated lung injury by triggering NLRP3-dependent pyroptosis in alveolar macrophages [J]. Biochim Biophys Acta Mol Basis Dis, 2020, 1866(5): 165685.

[49] ENDO K, WENG H, KITO N, et al. MiR-216a and miR-216b as markers for acute phased pancreatic injury [J]. Biomed Res, 2013, 34(4): 179-88.

[50] ZHU H, SONG Y, KONG X, et al. Expression of microrNA-216A in patients with acute pancreeatitis complicated with lung injury and its effect on endothelial cell permability [J]. J Chin Pancreas Dis, 2020, 20: 4.

[51] 朱惠云. 腺泡源性miR-216a通过exosome途径介导血管高通透性在重症急性胰腺炎肺损伤中的机制研究[D]. 上海: 中国人民解放军海军军医大学, 2019.

[52] SHI N, DENG L, CHEN W, et al. Is microRNA-127 a novel biomarker for acute pancreatitis with lung injury [J]? Dis Markers, 2017, 2017: 1204295.

[53] YING H, KANG Y, ZHANG H, et al. MiR-127 modulates macrophage polarization and promotes lung inflammation and injury by activating the JNK pathway [J]. J Immunol, 2015, 194(3): 1239-51.

[54] XIE T, LIANG J, LIU N, et al. MicroRNA-127 inhibits lung inflammation by targeting IgG Fcγ receptor I [J]. J Immunol, 2012, 188(5): 2437-44.

[55] AZIZ M, BRENNER M, WANG P. Extracellular CIRP (eCIRP) and inflammation [J]. J Leukoc Biol, 2019, 106(1): 133-46.

[56] YANG Q, LUO Y, LAN B, et al. Fighting fire with fire: exosomes and acute pancreatitis-associated acute lung injury [J]. Bioengineering, 2022, 9(11): 615.

[57] LIU W, REN Y, WANG T, et al. Blocking CIRP protects against acute pancreatitis by improving mitochondrial function and suppressing pyroptosis in acinar cells [J]. Cell Death Discov, 2024, 10(1): 156.

[58] XU Q, WANG M, GUO H, et al. Emodin alleviates severe acute pancreatitis-associated acute lung injury by inhibiting the cold-inducible RNA-binding protein (CIRP)-mediated activation of the NLRP3/IL-1β/CXCL1 signaling [J]. Front Pharmacol, 2021, 12: 655372.

[59] HU Q, ZHANG S, YANG Y, et al. Extracellular vesicle ITGAM and ITGB2 mediate severe acute pancreatitis-related acute lung injury [J]. ACS Nano, 2023, 17(8): 7562-75.

[60] KANTHASAMY K A, AKSHINTALA V S, SINGH V K. Nutritional management of acute pancreatitis [J]. Gastroenterol Clin North Am, 2021, 50(1): 141-50.

[61] SINGH N, SONIKA U, MOKA P, et al. Association of endotoxaemia & gut permeability with complications of acute pancreatitis: secondary analysis of data [J]. Indian J Med Res, 2019, 149: 763-70.

[62] SHAO Y, JIANG Y, WANG J, et al. Inhibition of circulating exosomes release with GW4869 mitigates severe acute pancreatitis-stimulated intestinal barrier damage through suppressing NLRP3 inflammasome-mediated pyroptosis [J]. Int Immunopharmacol, 2024, 126: 111301.

[63] SHAO Y, LI Y, JIANG Y, et al. Circulating exosomal miR-155-5p contributes to severe acute pancreatitis-associated intestinal barrier injury by targeting SOCS1 to activate NLRP3 inflammasome-mediated pyroptosis [J]. FASEB J, 2023, 37(6): e23003.

[64] TIAN R, WANG R L, XIE H, et al. Overexpressed miRNA-155 dysregulates intestinal epithelial apical junctional complex in severe acute pancreatitis [J]. World J Gastroenterol, 2013, 19(45): 8282-91.

[65] ZHANG L, SHEN J, CHENG J, et al. MicroRNA-21 regulates intestinal epithelial tight junction permeability [J]. Cell Biochem Funct, 2015, 33(4): 235-40.

[66] PARK E J, SHIMAOKA M, KIYONO H. Functional flexibility of exosomes and microRNAs of intestinal epithelial cells in affecting inflammation [J]. Front Mol Biosci, 2022, 9: 854487.

[67] MURAO A, AZIZ M, WANG H, et al Release mechanisms of major DAMPs [J]. Apoptosis, 2021, 26: 152-62.

[68] CHEN X, ZHAO H X, BAI C, et al. Blockade of high-mobility group box 1 attenuates intestinal mucosal barrier dysfunction in experimental acute pancreatitis [J]. Sci Rep, 2017, 7(1): 6799.

[69] LIU S, STOLZ D B, SAPPINGTON P L, et al. HMGB1 is secreted by immunostimulated enterocytes and contributes to cytomix-induced hyperpermeability of Caco-2 monolayers [J]. Am J Physiol Cell Physiol, 2006, 290(4): C990-9.

[70] BALA S, PETRASEK J, MUNDKUR S, et al. Circulating microRNAs in exosomes indicate hepatocyte injury and inflammation in alcoholic, drug-induced, and inflammatory liver diseases [J]. Hepatology, 2012, 56(5): 1946-57.

[71] HU J, HUANG S, LIU X, et al. miR-155: an important role in inflammation response [J]. J Immunol Res, 2022, 2022: 7437281.

[72] JIMÉNEZ-ALESANCO A, MARCUELLO M, PASTOR-JIMÉNEZ M, et al. Acute pancreatitis promotes the generation of two different exosome populations [J]. Sci Rep, 2019, 9(1): 19887.

[73] HU Y, YU Y. Dysregulation of miR-192-5p in acute pancreatitis patients with nonalcoholic fatty liver and its functional role in acute pancreatitis progression [J]. Biosci Rep, 2020, 40(5): BSR20194345.

[74] LÜ L L, FENG Y, WEN Y, et al. Exosomal CCL2 from tubular epithelial cells is critical for albumin-induced tubulointerstitial inflammation [J]. J Am Soc Nephrol, 2018, 29(3): 919-35.