

植物细胞外囊泡在肿瘤疾病中的研究进展

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摘要 植物细胞外囊泡(plant extracellular vesicles, PEVs)是由植物活细胞向外部环境分泌的一类被脂质膜包被的囊泡。这种囊泡包裹脂质、蛋白质、核酸等诸多生物信息分子,可作为治疗剂通过抑制肿瘤微环境,调控肿瘤代谢,抑制肿瘤增殖、转移,促进肿瘤细胞凋亡,抑制肿瘤血管生成,降低肿瘤耐药性,调节肠道菌群及抑制炎症反应等多种途径发挥抗肿瘤作用,也可作为药物载体递送小分子、核酸和蛋白质等抗肿瘤药物与其发挥协同治疗疾病的作用,提高抗肿瘤的治疗效果。该文阐明了PEVs的生物发生、分离方法及成分分析,重点总结了其作为药物和载体在肿瘤疾病中的研究进展,以期PEVs在肿瘤疾病领域中的研究提供理论参考,推动其在肿瘤疾病治疗中的实际应用。

关键词 植物细胞外囊泡; 生物发生; 分离方法; 成分分析; 肿瘤疾病; 研究进展

Research Progress of Plant Extracellular Vesicles in Tumor Disease

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Abstract PEVs (plant extracellular vesicles) are nanoscale bilayer vesicles spontaneously secreted by living plant cells. PEVs encapsulate lipids, proteins, nucleic acid and many other biological information molecules. It can be used as a therapeutic agent to play an anti-tumor role by inhibiting tumor microenvironment, regulating tumor metabolism, inhibiting tumor cell proliferation and metastasis, promoting tumor cell apoptosis, inhibiting angiogenesis of tumor cells, reducing drug resistance, regulating the intestinal flora and inhibiting inflammatory responses in various ways. It can also be used as a drug carrier to deliver small molecules, nucleic acids and proteins anti-tumor drugs to play a synergistic role in the treatment of tumor diseases and improve the therapeutic effect. In this paper, the biogenesis, separation methods and component analysis of PEVs, and the research progress of PEVs as drugs and carriers in tumor diseases are summarized in detail, in order to provide theoretical reference for the research of PEVs in the field of tumor diseases and promote its practical application in the treatment of tumor diseases.

Keywords plant extracellular vesicles; biogenesis; separation methods; component analysis; tumor disease; research progress

细胞外囊泡(extracellular vesicles, EVs)根据分泌方式、直径和生物物理性质等特征,通常可分为三类不同亚群:外泌体、微囊泡及凋亡小体。EVs

最初被认为只是细胞处理胞内废物的一种方式^[1],随着研究的深入,发现其内含蛋白质、脂质及RNA等多种功能性成分,介导细胞间信息交流^[2]。越来

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越多的研究发现,从无毒植物中也能分离出与动物结构和功能方面相似的EVs,被称为植物EVs(plant EVs, PEVs)。多项研究表明,PEVs能够表现出与原始植物相似的药理功能,发挥抗肿瘤的治疗作用。其也可作为药物载体,高效地将装载的抗肿瘤药物递送到目标细胞,从而最大限度地降低细胞毒性和脱靶效应,并与装载的药物发挥协同治疗作用。此外,PEVs的靶向性可通过工程化改造进一步增强,使其功能更具特异性。这些特性使得PEVs在肿瘤疾病中的治疗效果整体显著提升,展现出广阔的应用前景。因此,本文对近年来PEVs在肿瘤疾病中的研究进展进行综述,为后续研究奠定理论基础。

1 PEVs概述

1.1 生物发生

据目前研究,PEVs可能有三种发生途径:多泡体(multivesicular bodies, MVBs)途径、外囊阳性细胞器(exocyst-positive organelle, EXPO)途径及液泡途径(图1)。其中,MVBs途径被认为是PEVs发生的主要途径,在病毒感染过程中,与Tetraspanin-8及PEN1相关的腔内囊泡(intraluminal vesicles, ILVs)由MVBs途径分泌到细胞外环境^[3-4]。此外,POULSEN等^[5]在研究拟南芥与外囊体复合物相关的EXO70E2

蛋白定位时,发现了一种与EXO70E2共定位的细胞器——EXPO。EXPO在形态上与MVBs不同,双膜结构的EXPO在细胞内形成后与质膜融合,直接将单层膜囊泡释放到细胞外环境。液泡途径主要发生在植物防御真菌病原体的过程中。感染期间,液泡开始与质膜融合并释放防御蛋白,以对抗病原体的入侵^[6]。相较于其他两种途径,液泡途径研究较少。

1.2 分离方法

常见的分离方法主要包括差速离心法、密度梯度离心法、超滤法、尺寸排阻色谱法、聚合物沉淀法、电泳偶联透析法、免疫亲和法、微流控技术等。差速离心法是基于EVs与其他成分在密度和大小上的差异性,通过逐步提高离心转速及离心时间,将EVs从样品中分离出来的技术^[7]。密度梯度离心法是基于差速离心法的一项改进分离技术,通常将样品添加到不同浓度梯度的介质(如蔗糖或碘克沙醇)顶部,在超速离心之后,PEVs主要分布在30%~45%蔗糖密度层之间,通过收集对应的介质层,可得到纯度较高的PEVs^[7-8]。超滤法是基于分子或颗粒尺寸大小的分离技术,它借助具有特定孔径的超滤膜微孔道结构,对样品施加相应的压力,使溶剂和小分子物质通过滤膜,而使EVs类的大分子物质被滤膜截留,从而分离出不同尺寸的纳米颗粒^[9]。尺寸排阻色谱法

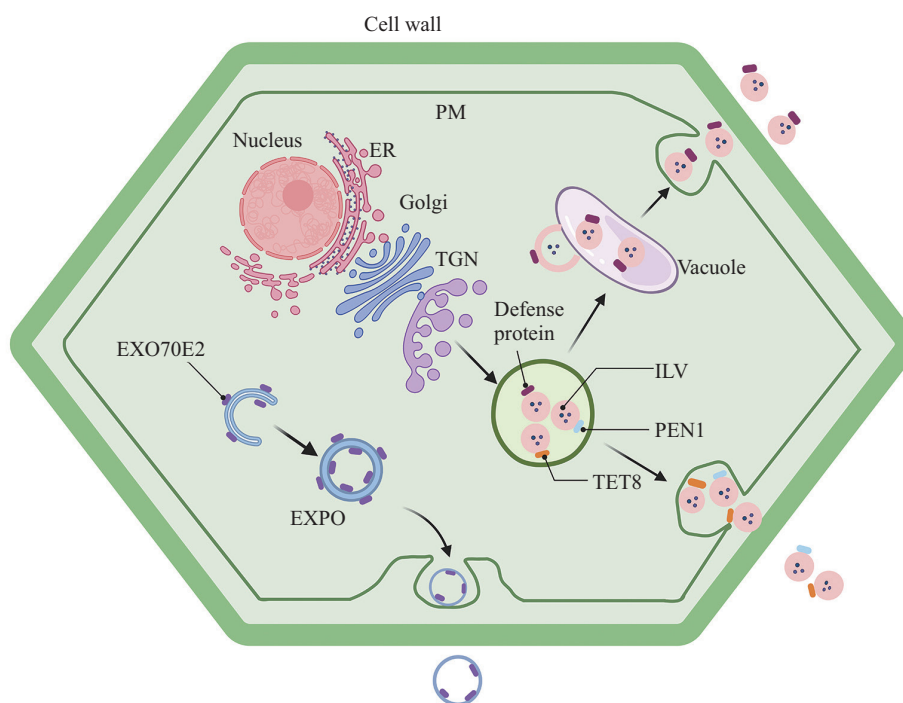


图1 PEVs的生物发生图(此图由Biorender平台绘制)

Fig.1 PEVs biogenesis graph (this graph is drawn by the Biorender platform)

与超滤法原理类似。色谱柱中填充具有多孔的凝胶颗粒, EVs类尺寸大于凝胶颗粒孔径的物质不能进入孔径, 通过凝胶颗粒之间的缝隙先被洗脱出来。而其他尺寸小于凝胶颗粒孔径的物质会进入凝胶颗粒内部通道, 因迁移路径延长而被较晚洗脱出来, 从而实现分离^[10]。聚合物沉淀法通常利用高分子聚合物, 如聚乙二醇(polyethylene glycol, PEG), 竞争性地结合游离水分子, 改变EVs的溶解性和分散性, 再通过低速离心使EVs沉降下来^[11]。电泳偶联透析法是一种利用EVs与透析膜孔径的不同, 结合电泳技术实现EVs分离的方法^[12]。免疫亲和法是一种基于抗原-抗体相互作用, 实现对EVs的特异性识别和捕获的方法, 是分离特异性EVs亚类最精确的方法^[13]。微流控技术是根据EVs的大小、形状等物理特征进行分离的方法^[14]。其中, 差速离心法与蔗糖密度梯度离心法联合使用是分离PEVs最常用的方法。这些分离方法各有优势和不足之处(表1)。因此, 从植物中分离出纯度较好、完整的EVs仍然具有挑战性, 目前尚无统一的分离提纯方法。需要根据具体的样本特性、后续实验对EVs纯度要求以及实验条件等

因素, 选择合适的分离提纯方法, 或者采用多种方法联合使用的策略, 以获得满足研究需求的EVs。

1.3 成分分析

PEVs包含多种成分, 如脂质、蛋白质、核酸以及小分子活性成分等(图2)。

1.3.1 PEVs中的脂质 脂质是PEVs骨架的主要组分, 目前常用薄层色谱(thin layer chromatography, TLC)和液相色谱-串联质谱(liquid chromatography-tandem mass spectrometry, LC-MS/MS)进行分析。PEVs中的脂质主要是磷脂和甘油, 包括磷脂酸(phosphatidic acid, PA)、磷脂酰胆碱(phosphatidylcholine, PC)、磷脂酰甲醇(phosphatidylmethanol, Pme)、磷脂酰乙醇(phosphatidylethanol, Pet)、单半乳糖基二酯甘油(monogalactosyldiacylglycerol, MGMG)、二乳糖基二酰基甘油(digalactosyldiacylglycerol, DGDG)和甘油三酯(triglyceride, TG)等^[17-18]。脂质在PEVs的摄取及内化过程中发挥着关键作用, 并且能够有效增强PEVs的穿透能力以及其内在治疗活性。如大蒜EVs中含有的PA与BV2细胞中的脑膜黏附信号蛋白1(brain abundant membrane attached signal protein

表1 PEVs分离方法的优缺点及其优化点比较

Table1 Comparison of the advantages, disadvantages and optimization points of PEVs separation methods

分离方法 Separation methods	优点 Advantages	缺点 Disadvantages	优化点 Optimization points	参考文献 Reference
Differential centrifugation	Technologically mature, wide application range, can be produced in large quantities	Products are susceptible to damage, low purity, requires specialized equipment and a large amount of time (>4 h)	Use pectinase and cellulase to treat samples before centrifugation ^[15] ; add a thin layer of high-concentration sucrose cushion at the bottom of the test tube ^[16]	[7]
Density gradient centrifugation	High product purity	Complex operation, long centrifugation time (>16 h), not suitable for large-scale production	None	[7]
Ultrafiltration	Relatively complete product structure, simple operation, short time-consuming	Low recovery rate	None	[9]
Size-exclusion chromatography	High product purity, relatively complete structure, short time-consuming	Low concentration	None	[10]
Polymer precipitation method	Simple operation, no need for specialized equipment, high yield	Low purity	pH4 or pH5	[11]
Electrophoresis-coupled dialysis method	High product purity, short time-consuming	Complex operation, limited separation efficiency	None	[12]
Immunoaffinity method	Strong specificity	Low yield, high antibody cost	None	[13]
Microfluidic technology	High efficiency, easy to carry, high degree of automation	Low purity	None	[14]

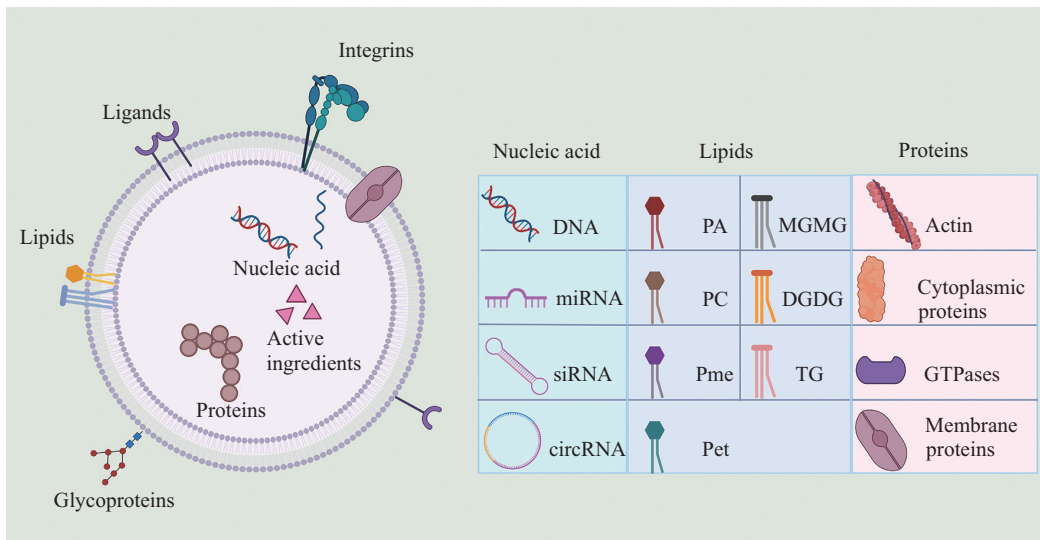


图2 PEVs的组成示意图(此图由Biorender平台绘制)

Fig.2 PEVs component graph (this graph is drawn by the Biorender platform)

1, BASP1)结合是其摄取所必需的^[19]。葡萄柚EVs中的PC有助于其优先被瘤胃球菌科细菌TSD-27吸收,促进了其从肠道到肝脏的迁移^[20]。人参EVs中的PC成分有助于其穿透血脑屏障^[21]。生姜EVs中的PA能够与牙龈卟啉单胞菌中的血红素结合蛋白35(heme binding protein 35, HBP35)发生结合,抑制牙龈卟啉单胞菌的增殖,从而对牙周炎起到积极的治疗效果^[22]。

1.3.2 PEVs中的蛋白质 PEVs中主要有肌动蛋白、胞质蛋白、GTPases(Rab蛋白质家族)及膜和囊泡相关的蛋白质等^[23]。大部分研究使用LC-MS/MS和聚丙烯酰胺凝胶电泳(sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE)分析EVs含有的蛋白质,通过对某些关键蛋白质进行定性及定量分析,并结合生物信息学技术对其功能进行深入研究,有助于揭示EVs的药理功能和相关疾病的发生机制。如CHEN等^[18]使用LC-MS/MS分析来研究茶叶外泌体中的蛋白质组成,共鉴定出446种蛋白质。进一步通过生物信息技术分析发现,有超过40种与癌症、免疫疾病和细胞成分相关的蛋白质,11种与细胞过程、代谢过程和催化活性通路相关的蛋白质,为相关疾病的进一步研究奠定了基础。动物源性EVs目前的标志蛋白可能是CD63、CD81、CD9跨膜蛋白家族和HSP90热休克蛋白家族^[24-25]。PEVs来源广泛,在此方面目前还未有统一的鉴定标准。有研究表明,突触融合素PEN1、ABC转运蛋白PEN3和

Tetraspanin-8可能是PEVs的候选表面标志蛋白^[26]。但目前未被广泛接受,在后续实践过程中还需进一步验证。

1.3.3 PEVs中的核酸 PEVs的核酸鉴定主要是RNA,主要包括小干扰RNA(small interfering RNA, siRNA)、微小RNA(microRNA, miRNA)及环状RNA(circular RNA, circRNA)等^[27]。其中,miRNA能跨越物种界限参与种间的基因表达调控,在疾病治疗中发挥着重要作用^[28]。大部分研究使用高通量测序及生物信息学技术来分析核酸。如YIN等^[29]通过高通量测序分析生姜中的miRNA谱,发现生姜外泌体中有27个表达水平较高的miRNA,进一步靶点预测和功能分析表明,这些miRNA主要参与炎症和癌症相关途径的调节。miRNA相关数据为PEVs的功能研究奠定了极大的基础,随着生物信息学的发展,组学分析在未来可能会揭示PEVs更多的药理作用。

1.3.4 PEVs中的小分子活性成分 PEVs中含有多小分子活性成分,在其发挥药理功能中具有关键作用。如葡萄柚EVs中有抗肿瘤作用的肌醇及抗菌、抗炎作用的桃叶珊瑚苷^[30]。草莓EVs中具有抗氧化的花青素、叶酸、黄酮醇和维生素C^[31]。黑桑叶EVs中有抗炎、抗肿瘤的芦丁、槲皮素3-O-葡萄糖苷(quercetin-3-O-glucuronide, Q3G)和山奈酚-3-O-葡萄糖苷(kaempferol-3-O-glucuronide, K3G)^[32]。槐米EVs中含有治疗脊髓损伤的关键成分芦丁^[33]。

2 PEVs抑制肿瘤的作用机制

PEVs作为治疗剂抑制肿瘤的作用机制见图3。

2.1 抑制肿瘤微环境

免疫抑制性肿瘤微环境(tumor microenvironment, TME)严重抑制了机体对肿瘤产生的免疫反应,进而减弱了基于免疫反应的肿瘤治疗效果。目前,研究发现PEVs可通过多种方式参与肿瘤的免疫调节过程,能够逆转TME中的免疫抑制。在肺癌小鼠模型中,青蒿衍生的EVs通过携带线粒体DNA(mitochondrial DNA, mtDNA)激活鸟苷酸-腺苷酸合成酶-干扰素基因刺激蛋白(cyclic GMP-AMP synthase-stimulator of interferon genes, cGAS-STING)通路,从而促进巨噬细胞从M2型(促肿瘤)极化到M1型(抗肿瘤),提高了PD-L1抑制剂(一种原型免疫检查点抑制剂)对小鼠肿瘤的治疗效果,并且增加了小鼠肿瘤内CD8⁺ T细胞(细胞毒性T细胞)的比例,从而增强了抗肿瘤免疫反应,加速了对肺癌细胞的清除^[34]。同样,人参EVs处理神经胶质瘤细胞可有效促进巨

噬细胞从M2型极化到M1型,并使TME中CD8⁺ T细胞比例显著增加,而CD4⁺ T细胞(辅助性T细胞)和调节性T细胞(regulatory T cells, Tregs)比例显著减少,且这不会损伤正常细胞^[21]。处理结肠癌细胞能够降低免疫检查点(PD-1、TIM3和ICOS)的表达水平,并促进更多的活化T细胞向TME募集^[35]。在近期的一项研究中,WANG等^[36]利用人参衍生的EVs与肿瘤细胞膜纳米颗粒(tumor membrane nanoparticles, TM-NPs)融合,制备了一种基于自体肿瘤抗原的个性化杂交膜纳米颗粒疫苗(hybrid membrane nanoparticles, HM-NPs)。EVs的融合可增强树突状细胞(dendritic cells, DCs)对自体肿瘤抗原的吞噬作用,促进DCs成熟,从而激活细胞毒性T淋巴细胞(cytotoxic T lymphocytes, CTLs),清除残留肿瘤细胞,抑制肿瘤复发和转移;在MB49膀胱癌模型中, HM-NPs可降低肿瘤复发率并延长小鼠的生存期。YANG等^[37]利用人参和菠菜衍生的外泌体样纳米囊泡制成了一种可注射的水凝胶,该水凝胶中的菠菜EVs部分与

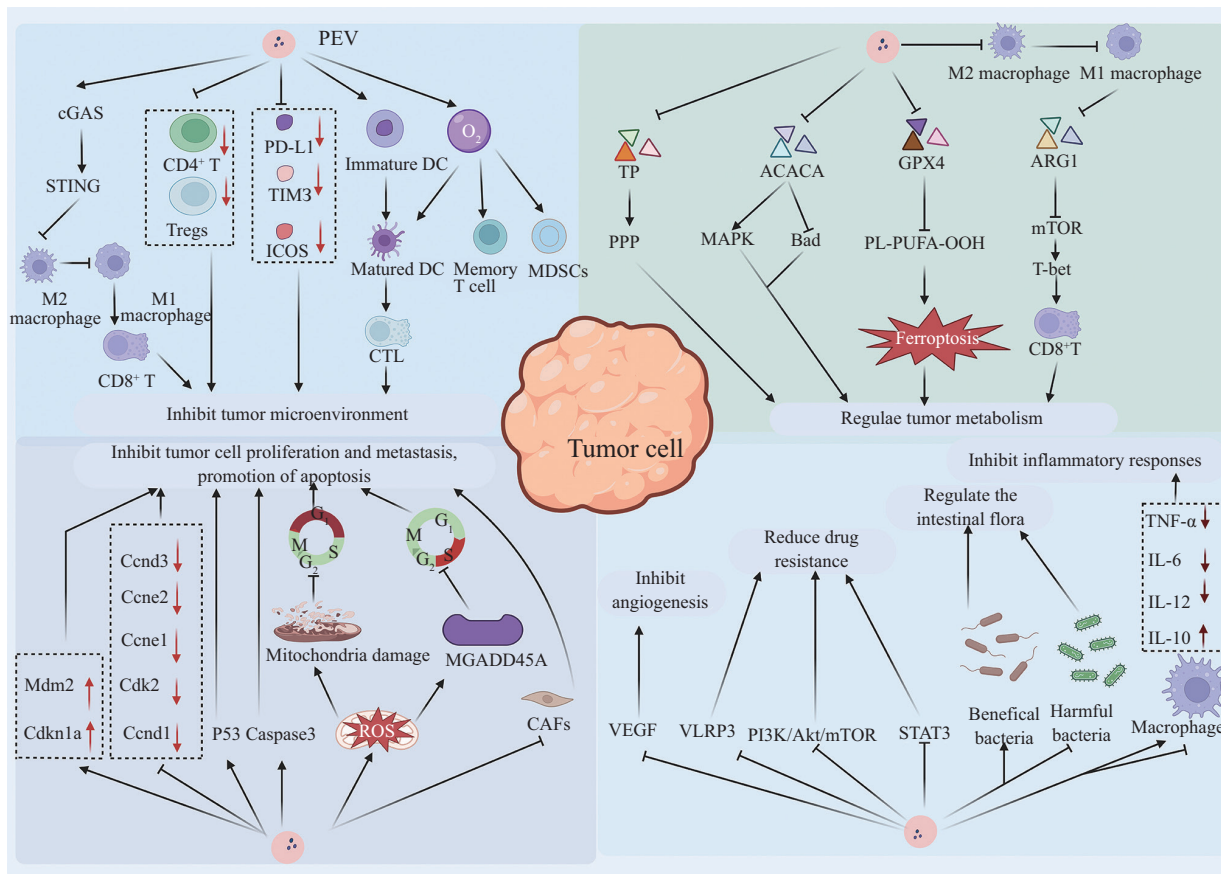


图3 以PEVs作为治疗剂在抑制肿瘤中的作用机制(此图由Biorender平台绘制)

Fig.3 The mechanism of action of PEVs as therapeutic agents in inhibiting tumors
(this graph is drawn by the Biorender platform)

马来酰亚胺修饰的OVA257-264肽(OVA-MaI)结合,形成SDEN@OVA复合物,从而催化氧气产生,缓解TME中缺氧诱导的免疫抑制,并促进DCs成熟和激活记忆T细胞,同时使骨髓源性抑制细胞(myeloid-derived suppressor cells, MDSCs)、Tregs和M2型巨噬细胞的比例显著降低,M1型巨噬细胞的比例显著增加,表明EVs可通过调节免疫功能来逆转TME的免疫抑制状态,对肿瘤表现出有效的治疗效果。总之,PEVs直接或间接参与调节TME,从而发挥治疗肿瘤疾病的作用。

2.2 调控肿瘤代谢

实际上,许多肿瘤与代谢有关,PEVs被证实可调控肿瘤代谢。人参纳米颗粒通过诱导胸苷磷酸化酶(thymidine phosphorylase, TP)表达下调,从而抑制磷酸戊糖途径(pentose phosphate pathway, PPP),减少PPP相关的能量生成和代谢中间产物,进一步抑制肺癌细胞(A549、NCI-H1299)的增殖和转移^[38]。柠檬EVs处理结直肠癌细胞抑制了参与脂肪酸从头合成的乙酰辅酶A羧化酶(acetyl-CoA carboxylase, ACACA)的活性,导致了脂肪酸合成受阻,从而通过抑制MAPK通路及激活B淋巴细胞瘤-2基因相关死亡激动蛋白(Bcl-2 associated agonist of cell death, Bad),抑制癌细胞的生长和增殖,发挥抗肿瘤作用,并且EVs处理对人骨髓基质细胞HS-5中ACACA的活性无影响^[39]。另外,铁死亡是一种铁依赖性、非凋亡途径的细胞死亡方式,是细胞膜上脂质过氧化物积累到一定程度,引发细胞死亡的结果,与肿瘤进展密切相关^[40]。谷胱甘肽过氧化物酶4(glutathione peroxidase 4, GPX4)是脂质过氧化作用的主要消除剂,在调节细胞铁死亡中发挥着重要作用^[41]。王智槟等^[42]研究发现石见穿外泌体可通过下调胱氨酸转运蛋白系统(system xc⁻, xCT)和GPX4表达,上调长链酰基辅酶A合成酶4(long-chain acyl-CoA synthase 4, ACSL4)表达,导致脂质过氧化物积累,从而诱导肝癌细胞发生铁死亡,发挥抗肿瘤作用。事实上,肿瘤代谢与肿瘤免疫也是相伴而行的,在结肠癌治疗中,人参来源的EVs能够重编程肿瘤相关巨噬细胞(tumor-associated macrophages, TAMs),促进巨噬细胞从M2型极化到M1型,抑制精氨酸酶-1(arginase-1, ARG1)的释放来维持TME中正常的L-精氨酸水平,随后激活动物雷帕霉素靶蛋白-T-bet(mammalian target of rapamycin-T-bet, mTOR-T-bet)通路,缓解TME

中的T细胞耗竭,下调免疫检查点(immune checkpoints, ICs)的表达并促进CD8⁺T细胞扩增,显著抑制肿瘤生长^[43]。总的来说,代谢重编程及失调现象伴随着肿瘤进展过程,这为其细胞的生长与分裂提供了关键的物质基础,PEVs能够改善此现象并发挥抗肿瘤作用。

2.3 抑制肿瘤增殖、转移,促进肿瘤细胞凋亡

PEVs能够有效抑制肿瘤细胞增殖、迁移和促进细胞凋亡。这可能受到多种调控因子的调节,如生长停滞和DNA损伤诱导蛋白45α(growth arrest and DNA damage-inducible 45 alpha, GADD45α)、线粒体膜电位及活性氧(reactive oxygen species, ROS)等。WANG等^[44]发现生姜EVs中存在细胞毒性成分姜酚和姜辣素,转录组学分析显示,其主要通过调控细胞周期相关基因(*Ccnd1*及*Cdkn1a/p21*等)和激活p53信号通路抑制黑色素瘤细胞B16F10的增殖并促进细胞凋亡。GAO等^[32]通过差速离心和密度梯度离心获得黑桑叶EVs,其能够通过半乳糖受体介导的内吞作用被肝癌细胞Hepa1-6优先摄取,并通过增加ROS的产生和触发线粒体损伤使细胞周期停滞在G₀/G₁期,抑制细胞增殖、迁移,诱导细胞凋亡。另外,金银花外泌体中的miR2911能够直接靶向SiHa、CaSki和HeLa三种HPV阳性宫颈癌细胞的*E6/E7*基因,抑制其蛋白表达。这一过程激活了p53和Caspase3信号通路,进而诱导了癌细胞凋亡^[45]。YANG等^[46]发现胃癌细胞(AGS、BGC-823和SGC-7901)经柠檬EVs处理后,柠檬EVs诱导了ROS的生成,从而使GADD45α过表达导致细胞周期停滞在S期和胃癌细胞凋亡,减少了肿瘤的发生。ANUSHA等^[47]揭示了生姜外泌体通过增加三阴性乳腺癌细胞MDA-MB-231内ROS水平、诱导线粒体膜电位下降及促进细胞膜上的磷脂酰丝氨酸易位等多种机制,抑制细胞增殖并促进细胞凋亡;同时,生姜外泌体还具有抑制癌细胞迁移和集落形成的能力,发挥抗肿瘤转移作用。另外,KIM等^[48]发现朝鲜树参树干汁液衍生的EVs能够对促肿瘤细胞侵袭和迁移有关的癌症相关成纤维细胞(cancer-associated fibroblasts, CAFs)产生浓度依赖性的抑制作用,这种抑制作用是通过降低生长因子和细胞外基质(extracellular matrix, ECM)相关基因的表达水平实现的,从而发挥抗肿瘤转移的作用。

2.4 抑制肿瘤血管生成

肿瘤增殖依赖于新血管的生成,这一过程与血

管内皮细胞的分裂、增殖和迁移有关^[49]。血管内皮生长因子(vascular endothelial growth factor, VEGF)是一种关键的血管生成因子,能够促进血管内皮细胞的增殖和存活,进而促进新血管的生成。PEVs能够携带抗血管生成因子,抑制TME中的血管生成。鸦胆子衍生的EVs通过抑制静脉内皮细胞HUVECs分泌VEGF以及降低细胞间的连接数量和血管长度,抑制血管的生成,从而发挥抗肿瘤作用;稳定性实验表明,经过EVs处理后,其抗血管生成活性在治疗一年后仍然保持不变^[50]。此外,大蒜衍生的EVs作用于人肾癌细胞A498及人非小细胞肺癌细胞A549能使VEGF的表达水平显著降低,并且对人皮肤成纤维细胞HDF无毒性^[51]。在慢性粒细胞白血病中,柠檬衍生的EVs不仅通过肿瘤坏死因子相关凋亡诱导配体(tumor necrosis factor-related apoptosis-inducing ligand, TRAIL)介导肿瘤细胞凋亡,而且还通过降低促血管生成的血管内皮生长因子-A(vascular endothelial growth factor-A, VEGF-A)、IL-6和IL-8的水平来抑制肿瘤生长^[52]。

2.5 降低肿瘤耐药性

某些研究发现,PEVs能够降低肿瘤细胞对药物的耐药性。如YANG等^[12]揭示了苦瓜EVs与5-氟尿嘧啶(5-fluorouracil, 5-FU)联合使用能够对口腔鳞状细胞癌细胞OSCC产生协同治疗效果。其RNA能够通过抑制核苷酸结合寡聚化结构域样受体蛋白3(nucleotide-binding oligomerization domain-like receptor protein 3, NLRP3)表达降低癌细胞对5-FU的耐药性,减少药物外排。类似地,CAO等^[53]揭示了西兰花EVs通过抑制PI3K/Akt/mTOR信号通路的异常激活,逆转结直肠癌细胞HT-29对5-FU的耐药性。此外,CHEN等^[54]证实了黄瓜EVs中的葫芦素B(cucurbitacin B, CuB)能够抑制信号转导和转录激活因子3(signal transducer and activator of transcription 3, STAT3)的磷酸化,有助于降低非小细胞肺癌细胞A549对STAT3信号通路依赖性抗癌药物的耐药性。

2.6 其他机制

另外,肠道菌群失衡与炎症反应也会驱动肿瘤的发生、生长和转移^[55-56],PEVs也可通过调节肠道菌群及抑制炎症反应缓解肿瘤的发生与发展。如YANG等^[57]证实了桔梗EVs治疗能够提升肿瘤小鼠肠道菌群的丰度及多样性,通过增加有益菌/有害菌

的比例,调整菌群失衡状态,增强免疫系统的功能,从而抑制三阴性乳腺癌生长。ZU等^[58]揭示了茶叶EVs处理RAW 264.7巨噬细胞在一定范围内会时间依赖性地抑制RAW 264.7巨噬细胞分泌促炎细胞因子TNF- α 、IL-6和IL-12,并促进抗炎细胞因子IL-10的分泌;在小鼠结肠肿瘤模型中,EVs治疗会显著减少小鼠的肿瘤数量,表明其可有效缓解炎症并抑制肿瘤的发展。

3 PEVs作为载体在肿瘤疾病中的应用

传统药物递送载体(如合成纳米颗粒、脂质体递送药物等)穿透生物屏障的能力较弱,免疫原性强,毒性大,且在靶向递送和生物相容性方面也表现不佳^[59]。PEVs由于来源于天然物质,具有优异的稳定性,能够耐受多种酶消化,抵抗极端pH环境,可将内含物稳定运送至受体细胞,使递送的药物分子表现出更好的稳定性和摄取靶向性,并延长药物在病变部位的半衰期和降低药物对机体的副作用^[15]。同时,对PEVs还可以进行工程化改造,使其功能更具特异性。因此,PEVs被视为是良好的药物递送载体,能够递送抗肿瘤药物,使其发挥更高效和特异的治疗效果。

3.1 PEVs递送小分子药物

刘洋^[60]利用生姜外泌体作为纳米载体,通过共孵育方法将紫杉醇药物封装在其内部,这一策略能够降低药物释放速率,延长药物在体内的作用时间,从而显著提升治疗宫颈癌的效果。LU等^[61]将抗肿瘤药物阿霉素(doxorubicin, DOX)封装到芹菜外泌体样纳米囊泡中,其可以响应特定的外界刺激,如pH变化,从而释放负载的DOX药物,这种刺激响应性释放机制能够确保药物在肿瘤部位的有效浓度,使其更高效地抑制肿瘤细胞增殖及调节TME中的免疫细胞、血管生成。在另一项研究中,NIU等^[62]设计了一种新的基于葡萄柚EVs与载有DOX的肝素基纳米颗粒(doxorubicin-loaded heparin-based nanoparticles, DNs)结合的仿生药物传递系统;与传统的PEVs封装相比,此递送系统实现了前所未有的4倍药物负载能力,被证明能够最大限度地增强脑肿瘤对药物的摄取,并显著提高了其治疗神经胶质瘤的疗效。XIAO等^[63]通过将功能性肝素-环状RGD肽修饰到柠檬EVs表面,并进一步装载DOX,使得柠檬EVs纳米药物能够有效进入耐药癌细胞,其多样化的内吞能

力可耗散细胞内能量,减少ATP产生,从而抑制药物外排,表现出优异的抑制耐药癌细胞增殖的能力,有效克服卵巢癌多药耐药性。

3.2 PEVs递送核酸药物

核酸治疗药物主要在细胞内发挥作用,但通常对体内的核酶敏感,且不能靶向到达病变的组织或器官^[64]。PEVs可被用于递送核酸药物发挥抗肿瘤作用,如miRNA及siRNA。CORVIGNO等^[65]发现将mi-R146a-5p加载到西瓜衍生的EVs与聚酰胺-胺型树枝状高分子(polyamidoamine dendrimers, PAMAM)结合的创新递送系统中后,miR146a-5p即会直接降低与血管形成和血管完整性相关基因的表达水平,从而降低血管形成活性;这又会间接降低刺激血管形成的分泌蛋白的表达水平,从而发挥抗肿瘤效果。此递送方式利用了PAMAM的高效装载效率和西瓜EVs的生物相容性及靶向性,从而提高了肿瘤的治疗效果。此外,HUANG等^[66]报道了一种食用且不含阳离子的猕猴桃EVs介导的表皮生长因子受体(epidermal growth factor receptor, EGFR)靶向siRNA递送系统,该系统能够抑制多药耐药性肺癌。

3.3 PEVs递送蛋白质药物

PEVs也可作为蛋白质的输送载体,目前的研究主要集中在递送结合肿瘤抗原的热休克蛋白70(heat shock protein 70, HSP70)分子中。HSP70作为一种重要的应激蛋白,不仅会激活机体适应性和先天性抗肿瘤免疫反应,还在克服肿瘤耐药性等方面展现出巨大潜力^[67]。但部分肿瘤细胞对HSP70的免疫调节作用不敏感,并且未经装载的HSP70蛋白难以精准地到达肿瘤细胞及易被体内蛋白酶降解,不能维持有效的治疗剂量^[68]。PEVs具有良好的生物相容性及脂质双分子层结构,能够有效避免HSP70被免疫系统的清除及蛋白酶的降解,使HSP70靶向到达肿瘤细胞部位。KILASONIYA等^[69]将HSP70装载到葡萄柚分离的外泌体中,使其靶向递送到神经胶质瘤细胞,增强了胶质瘤细胞对化疗药物的敏感性,抑制了其增殖,从而在细胞内发挥高效的抗肿瘤效果。类似地,GARAEVA等^[68]指出葡萄柚衍生的EVs装载的HSP70与20倍量的游离形式的HSP70具有相同的抗肿瘤作用,在体外该EVs装载的HSP70有效激活了对结直肠癌细胞的先天抗肿瘤免疫,刺激了特异性抗肿瘤CD8⁺T细胞积累;在小鼠结肠癌模型中,此EVs装载药物的处理能够显著延长动物生存期,

抑制肿瘤生长,并显著降低血浆中促肿瘤细胞因子TGF- β 1和IL-10数量。未来,可通过设计蛋白质使其靶向细胞膜上的脂质筏,从而在PEVs质膜上形成聚集体,增加其被包裹进PEVs的面积和概率,并引入选择性脂质标签实现基因表达调控,有望进一步提升其在肿瘤治疗中的效果^[70]。

4 结论与展望

近年来,PEVs在肿瘤疾病中的研究取得了显著进展。关于PEVs作为治疗剂发挥抗肿瘤作用,已经阐明了多种作用机制,大多数研究指出其与抑制肿瘤微环境和抑制肿瘤细胞增殖、迁移和促进肿瘤细胞凋亡有关,少数研究指出其与调控肿瘤代谢、抑制肿瘤血管生成、降低肿瘤耐药性、调节肠道菌群及抑制炎症反应有关。一种PEVs可能通过多种机制在特定类型的肿瘤中发挥抗肿瘤作用,或者在不同类型的肿瘤中通过不同的机制发挥治疗作用。另外,PEVs还可作为载体,递送包括小分子、核酸、蛋白质在内的多种抗肿瘤药物,实现活性成分的共同靶向转运,从而协同治疗疾病。

尽管如此,PEVs的研究目前仍处于初步阶段。主要挑战集中在样本量较小及分离技术的局限性,若要将其进一步应用于临床中,首先要实现的是探索一种高效、简便的标准化提取分离技术,同时确保PEVs的高产量与稳定性。如可先使用植物细胞培养或组织培养技术获得持续、稳定性一致的原材料^[15],再通过基于快速疏水相互作用色谱(hydrophobic interaction chromatography, HIC)技术的毛细血管通道聚合物(capillary-channelled polymer, C-CP)尖端分离方法高效获得较纯的PEVs^[71],并与TMO或海藻糖溶液在低温下储存使其表现出更高的稳定性和蛋白质含量以便用于临床中^[72-73]。为解决这些问题并推动该领域标准化进程,国际细胞外囊泡学会(International Society for Extracellular Vesicles, ISEV)可组建一个新的工作组,专注于制定一套统一的PEVs命名、分离与储存、表征(包括PEVs形态、粒径、电位、成分分析及表面标志物等)、药物装载方法、体内给药途径及质量控制标准化等方面的具体指标和操作模式,以确保不同的实验结果具有可比性,并提高研究结果的准确性和可靠性,避免治疗效果的不一致,为肿瘤的临床治疗提供坚实的基础并使监管简单化。

另外,目前对于PEVs在肿瘤中发挥作用的相关机制尚不完全清楚,后期可利用多组学技术全面分析PEVs的成分和功能,揭示其在肿瘤治疗中的具体机制。随着生物技术的不断进步,在未来应该加强基础研究,探索PEVs与装载药物的结合机制及可装载药物类型(如通过分子对接预测药物与PEVs膜上特定蛋白质或脂质分子的潜在结合位点,并通过表面等离子共振及实验进行验证),在此基础上结合工程化技术,针对其结合机制进行优化,以提高装载药物效率并拓展可装载药物类型。后期对PEVs的研究重点可能集中于其作为药物载体对其主动定向修改(如表面修饰和功能基团插入)和刺激(如pH、温度、光等)响应两方面^[61,74-76],并有望与传统治疗手段相结合,实现对肿瘤的高效靶向治疗。值得注意的是,PEVs目前仍处于发展阶段,在大规模生产使用前要充分评估其安全性和有效性(包括毒理学实验、药代动力学研究和临床试验等),确保获得伦理审查委员会的批准,严格遵守国家相关法律法规。此外,在长期治疗过程中,还需评估PEVs可能在动物或人类体内蓄积的不良反应,并在不影响治疗效果的同时,通过调整用药量或服药间隔,最大限度地减少PEVs在体内的积累。尽管存在上述挑战,但我们相信PEVs在未来将具有重要的肿瘤临床应用价值。

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