外泌体在肿瘤诊断与治疗中的研究进展

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摘要 外泌体是由细胞分泌的大小为30~130 nm, 可携带供体细胞的一些生物学特性的转运囊泡, 其包含的蛋白质、RNA等多种生物活性分子在正常生理代谢反应、肿瘤等疾病的发生发展中发挥着重要的作用, 是细胞间信息传递和物质交换的重要媒介。作为新型的天然内源性物质转运载体, 外泌体具有低毒性、低免疫原性、渗透性好等优势。目前外泌体已成功负载小分子化学药物、基因等生物活性分子, 以用于肿瘤的诊断与治疗。该文基于外泌体的生物学特性、作为诊断性生物标志物和药物载体的优势, 以及在肿瘤的诊断与治疗的应用等方面进行综述。

关键词 外泌体; 药物载体; 肿瘤; 诊断; 治疗

Research Progress of Exosomes in the Diagnosis and Treatment of Tumors

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Abstract Exosomes are transport vesicles with a size of 30-130 nm, which are secreted by cells and possessed the biological characteristics of donor cells. As an important medium for information transmission and material exchange among cells, exosomes contain proteins, RNA and other biologically active molecules and play an important role in the development of normal physiological metabolic reactions, tumors and other diseases. As a new natural endogenous material transport carrier, exosomes have the advantages of low toxicity, low immunogenicity, and good permeability. Currently, exosomes have been successfully loaded with small molecule chemical drugs, genes and other biologically active molecules for the diagnosis and treatment of cancer. This article reviews the biological characteristics of exosomes, the advantages of exosomes as diagnostic biomarkers and drug carriers, and the application in tumor diagnosis and treatment.

Keywords exosomes; drug carrier; tumor; diagnosis; treatment

肿瘤是威胁人类健康的主要杀手之一, 如何更好地防治肿瘤一直是研究者们关注的热点[1]。外泌体是由细胞分泌产生的纳米级囊泡, 在肿瘤的发生发展、诊断和治疗方面发挥着重要作用[2]。一方面, 从血液、尿液、脑脊液和唾液等体液中分离得到的外泌体, 其内含物中包含能反映供体细胞状态的物质, 因此外泌体可能作为多种疾病特别是肿瘤诊断的生物标志物[3]。另一方面, 外泌体以其天然的物质转运特性、相对较小的分子结构和优良的生物相容性, 可作为优良的药物载体[4]。通过生物技术方法可将外泌体改造成靶向性药物载体, 协助生物大分子或抗肿瘤药物发挥抗肿瘤作用, 从而达到高效低毒杀伤肿瘤的效果[5]。该文总结了外泌体的生物学特性, 以及作为诊断性生物标志物和药物载体在肿瘤

收稿日期: 2018-09-07 接受日期: 2018-11-13
国家自然科学基金(批准号: 81872497)和陕西省自然科学基金(批准号: 2017JM8004)资助的课题
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Received: September 7, 2018 Accepted: November 13, 2018
This work was supported by the National Natural Science Foundation of China (Grant No.81872497) and the Natural Science Foundation of Shaanxi Province (Grant No.2017JM8004)
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网络出版时间: 2019-04-02 12:42:01 URL: http://kns.cnki.net/kcms/detail/31.2035.Q.20190402.1241.006.html

的诊断与治疗方面的应用, 为肿瘤诊疗一体化提供理论依据。

1 外泌体的生物学特性

外泌体是一类由细胞分泌产生的含有复杂RNA和蛋白质的小膜泡[2]。1983年, Johnstone等[6]在研究未成熟红细胞向成熟红细胞转化时, 从羊红细胞上清液中分离到一种小囊泡, 最初被认为是细胞分泌的垃圾, 是没有用的东西, 后来发现, 这些小囊泡包含有很多RNA成分, 还可以向靶细胞传递信息, 这才受到人们的重视。外泌体的产生与细胞膜内陷相关, 细胞内膜向内凹陷形成含有多个小囊泡的多泡体(multivesicular body, MVB), 其中包裹不同的功能性物质如蛋白质、mRNA和miRNA等。MVB可进一步与溶酶体融合后降解, 或者可以在与质膜融合后释放腔内囊泡进入细胞外空间, 其中的囊泡释放到细胞外即为外泌体[7]。

进一步研究发现, 外泌体是直径为30~130 nm的球形结构, 其包含供体细胞的蛋白质、脂质、mRNA、miRNA、小的非编码RNA以及基因组DNA等生物活性物质, 这些被包裹的生物大分子可以在靶细胞中发挥一系列作用, 是细胞间接通讯的重要媒介[8]。外泌体的一个关键功能是将其内含物从供体细胞转运到受体细胞, 使受体细胞的基因和表型修饰。目前认为, 外泌体从供体细胞转运到受体细胞主要有三种可能的途径(图1)[9]: (1)外泌体通过受体–配体相互作用或脂质(如磷脂酰丝氨酸)与靶细胞相互作用; (2)外泌体与受体细胞的质膜融合, 随后将外泌体载体释放到受体细胞的胞质中; (3)外泌体通过内吞作用或转胞吞作用内化到受体细胞中。

此外, 外泌体的内源性使其具有生物相容性好及免疫原性低的特点, 较小的尺寸允许它们穿过生理病理屏障将其携带的物质递送到下面的组织, 而且还可以通过体内或体外的修饰, 装载靶基因或药物分子。基于这些特性, 越来越多的人关注外泌体在疾病特别是肿瘤诊疗领域的研究。

2 外泌体作为肿瘤诊断性生物标志物的研究

外泌体可以从多种体液获得, 包括血液、母乳、羊水、唾液、恶性腹水和尿液等[10]。细胞在正常和

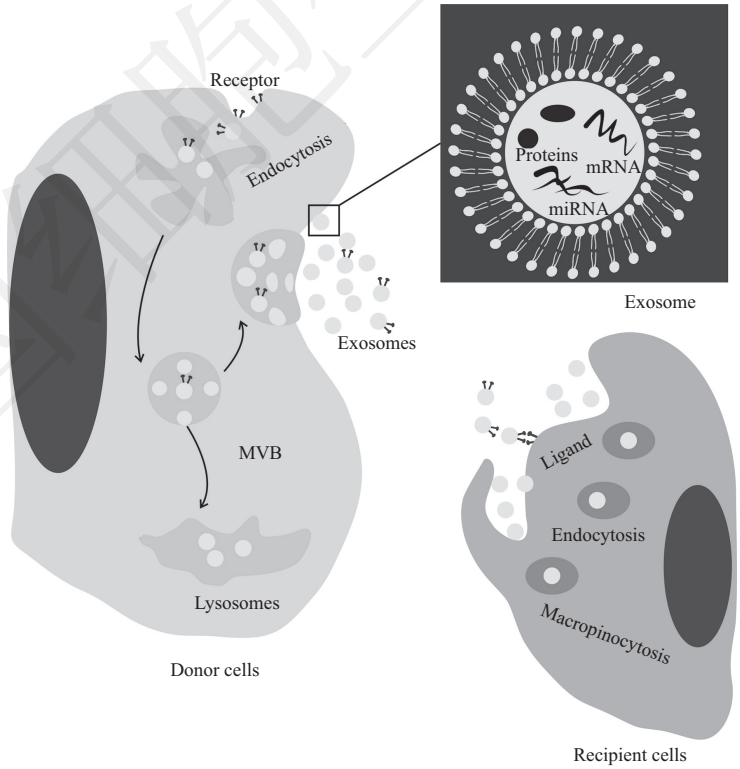


图1 外泌体从供体细胞到受体细胞的转运

Fig.1 Transportation of exosomes from donor cells to recipient cells

病理条件下都可以分泌外泌体。目前常用的外泌体提取方法有很多种, 在这里, 我们总结了常见的分离方法及其优缺点和局限性[11](表1)。外泌体携带的核酸和蛋白质等生物信息可以反映供体细胞的生理或病理状态, 因此被广泛认为是很有价值的生物标志物, 在实验和临床诊断中有很好的应用潜力。

从体液中获取外泌体是一个相对简单的过程, 因此越来越多的研究者开始关注将外泌体作为肿瘤或其他疾病的诊断标志物。有研究报道, 与正常细胞相比, 肿瘤细胞分泌更多的外泌体[12]。外泌体标记物CD63属于四跨膜蛋白家族, 与正常人相比, 黑素瘤患者血浆中CD63丰度显著增加。研究者对各种类型的人类癌症中的外泌体的标志性蛋白质进行了比较分析, 结果显示, 源自恶性癌细胞的外泌体比源自正常细胞系的外泌体中的CD63含量高, 证明了外泌体中CD63可以作为癌症诊断的生物标志物[13]。食管鳞状细胞癌(esophageal squamous cell carcinoma, ESCC)患者血清中外泌体中的miRNA-21浓度高于没有全身炎症的良性肿瘤患者, 表明外泌体中miRNA-21也可作为诊断食管鳞状细胞癌的标志物[14]。也有研究报道, 在ESCC患者的血清样本中分离的外泌体中miRNA-1246的浓度升高, 但在ESCC组织样本中未上调[15]。这一发现表明, 循环外泌体中miRNA-1246可作为ESCC中新的诊断和预后生物标志物。此外, 尿液外泌体蛋白也作为膀胱癌和前列腺癌的潜在的生物标志物而被研究。研究者通过比较膀胱癌患者和健康者的尿液外泌体的蛋白质组学特征确定潜在的膀胱癌外泌体生物标志物, 包括8种尿液外泌体蛋白质(5种与表皮生长因子受体途径相关的蛋白质、Gs蛋白质的α亚基、抵抗素和视黄酸诱导蛋白3)。从前列腺癌患者收集的尿液外泌体中也证实了两种已知的前列腺癌生物标志物PCA-3和TMRPRSS2:ERG[16]。近期, Srivastava等[17]研究发现, 外泌体可作为子宫内膜癌(endometrial cancer, EC)的诊断和预后生物标志物。他们评估了EC患者和有EC症状但没有确诊的患者的尿液来源外泌体中独特的miRNA表达谱, 发现EC患者尿液外泌体中hsa-miR-200c-3p富集最多, 说明外泌体中miRNA的差异可用于发现生物标志物特征和EC诊断。

到目前为止, 外泌体作为肿瘤诊断生物标志物的研究仍处于早期发展阶段, 但已展现出广泛的应用价值和潜力。

3 外泌体作为肿瘤治疗工具的研究

外泌体作为天然内源性纳米级囊泡, 可作为理想的药物递释系统来携载生物大分子或其他化学药物, 在肿瘤靶向治疗领域发挥重要的优势。

3.1 外泌体自身作为治疗工具的研究

一些细胞分泌的外泌体具有特定的生物学功能, 如间充质干细胞(mesenchymal stem cells, MSCs)、树突细胞(dendritic cells, DCs)和T细胞等分

表1 外泌体提取方法的比较(根据参考文献[11]修改)

Table 1 Comparison of methods for extraction of exosomes (modified from reference [11])

方法 Methods	优点 Advantages	缺点 Disadvantages	参考文献 References
Ultracentrifugation	Current gold standard, large quantity, low cost	Time-consuming, low recovery and purity	[18]
Density gradient centrifugation	Established protocol, high and purity	Large sample volume, low extraction rate	[19-20]
Polymerization precipitation	High extraction rate, easy and user-friendly processing	Difficulty in scaling, lack specificity	[21]
Mmunomagnetic bead separation	Simple operation, high specificity	Affect biological activity	[22]
Size-exclusion chromatography	Wide variety of eluents, high yield	Need special equipment, lack specificity	[23]
Field flow fractionation	Broad separation range, large quantity	Requires fractionation, equipment, time-consuming	[24]
Kit extraction	Fast, easy operation	The application has limitations, expensive	[25]

泌产生的外泌体具有作为治疗工具的潜力。

有研究证实, MSCs来源的外泌体具有一定的疾病治疗作用。MSCs来源的外泌体能够重现其供体细胞的免疫调节和细胞保护活性, 携带的miRNA-146b还能有效抑制胶质瘤细胞生长[26-27]。此外, 骨髓MSCs衍生的外泌体在心肌缺血、再灌注损伤、缺氧诱导的肺动脉高压和脑损伤模型中具有很好的保护作用[28-30]。在乳腺癌研究中, MSCs衍生的外泌体通过血管内皮生长因子下调和miR-16转移在小鼠模型中有效抑制乳腺癌细胞生长[31]。类似地, MSCs外泌体的miRNA可以促进乳腺癌细胞在转移过程中保持休眠状态[32-33]。

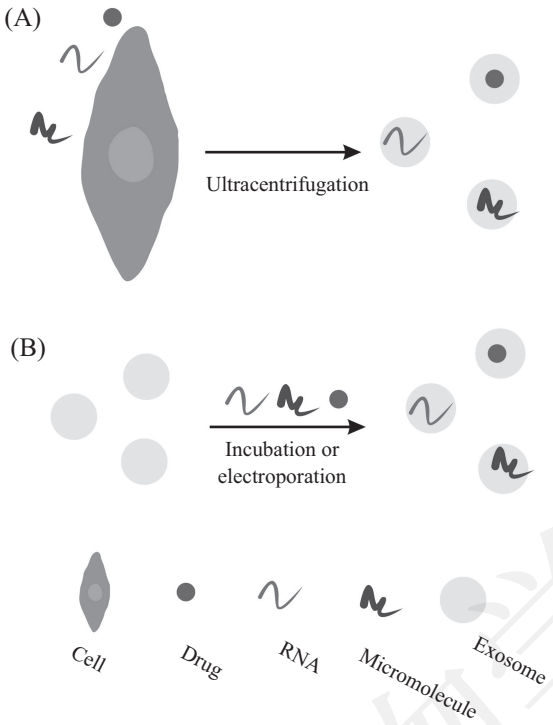
DCs衍生的携带H-Y肽的外泌体在体内可激活特异性免疫T细胞, 而有趣的是, 外泌体不能在体外激活特异性免疫T细胞, 除非存在成熟的DCs[34]。肿瘤细胞来源的外泌体可将携带的肿瘤相关抗原转运至DCs, DCs摄取抗原后, 引发$CD8^+$ T细胞依赖的抗肿瘤免疫效应[35]。而DCs来源的外泌体可以与抗原MHC分子结合, 形成抗原抗体复合物, 使成熟的DCs刺激T细胞增殖来引起免疫反应[36]。

B细胞来源的外泌体则是直接与MHC I、MHC II、共刺激因子和黏附因子结合, 刺激T细胞增殖引起免疫应答。此外, 调节性T细胞(regulatory cells, Treg)是T细胞的亚群, 维持自身免疫耐受并限制其他免疫应答, Treg免疫调节功能的一个关键目标是DCs。Treg衍生的外泌体携带的miRNA转移到DCs, 特别是两种miRNA, 即miR-150-5p和miR-142-3p, 当它们与Treg和Treg衍生的外泌体相互作用后, 在DCs中增加[37]。这些结果表明, 通过外泌体进行的miRNA的细胞间转移可能是Tregs调节DCs功能的新机制, 并且可能代表抑制组织中免疫反应的机制。

3.2 外泌体作为药物载体的研究

外泌体作为药物载体的优势在肿瘤治疗研究中, 如何将抗肿瘤药物精准递送到病灶部位一直是研究者努力的方向, 因此各种药物载体已被研发, 如脂质体、胶束、微泡等, 但这些载体本身存在许多弊端, 如免疫排斥、载药率低、靶向性差等。理想的药物载体要能够逃避宿主免疫系统, 实现精准靶向递药, 无毒且有足够长的半衰期等。而外泌体作为内源性药物载体, 与传统化学方法合成的药物载体相比具有诸多优势: (1)从血液、尿液、唾液等[38]多种体液获得的外泌体, 具有良好的生物相容性及稳定性, 可以极大地降低自身免疫排斥反应, 减少对病人造成的额外伤害。(2)外泌体在体内半衰期较长, 药物亦可在体内保持一个较长的半衰期从而能够提高疗效。(3)药物递送的主要障碍是透过生理病理屏障, 特别是血脑屏障(blood-brain barrier, BBB), 外泌体通过被动或主动靶向策略, 促使治疗性药物穿过血脑屏障实现药物的靶向递送, 这为全身向脑部输送药物提供了机会。(4)药物载体中使用的纳米载体的大小决定其血液循环过程的稳定性, 直径在30~130 nm的外泌体, 一方面可以避免被肾脏迅速排出、清除, 另一方面也可以避免被网状内皮系统吞噬、吸收。(5)不同来源的外泌体具有定向归巢能力, 例如源自间充质干细胞的外泌体可以趋向于炎症部位, 且具有抗炎作用; 黑色素瘤的外泌体可靶向于淋巴结, 以促使肿瘤的发生及转移[39]。(6)与传统药物载体相比, 外泌体可以分别在体内和体外加载药物。体内载药主要指将药物转入供体细胞进而获取已装载有目的药物的外泌体; 体外载药是指先获取外泌体, 再将药物通过电转或孵育等方式加载到外泌体中(图2)[40]。(7)外泌体不仅可以包载亲脂性药物, 还可以高效包载亲水性药物, 如外泌体可以很好地亲和核酸类药物, 极大地提高了载药率[41]。

尽管外泌体在分离、提取和纯化等方面还存在很大挑战, 但已经有许多报道证明其作为治疗性药物载体在肿瘤治疗方面的潜力。目前, 有多种不同形式的外泌体被研究开发装载小分子药物用于肿瘤的治疗。外泌体表现出细胞依赖性药物递送, 并且其生物分布取决于亲本细胞。因此, 利用外泌体包载药物需要考虑外泌体的来源、提纯方法、载药方式、载药种类以及最后的载药递释系统的使用方式等[42-43]。用来源于细胞系HEK-293的外泌体包载与尿嘧啶磷酸核糖基转移酶融合的自杀基因胞嘧啶脱氨酶, 在加入前体药物5-尿胞嘧啶时能够诱导神经鞘细胞死亡, 通过每周向实体瘤内注射外泌体, 两个月后发现肿瘤体积显著变小[44]。类似地, 利用小鼠成纤维细胞L929细胞来源的外泌体包载针对转化生长因子-β1(transforming growth factor-β1, TGF-β1)的siRNA递送至肉瘤细胞, 在小鼠皮下瘤模型中, 注射5次载有TGF-β1 siRNA的外泌体后, 能够显著抑制肿瘤中TGF-β1的表达和下游信号的传导, 并且抑制了原发性肿瘤生长以及肺转移的发生; 且剂量很低的siRNA(每次注射4×10^{-4} nmol)时就达到了很好的



A: 通过转染供体细胞装载外泌体; B: 外泌体分离之后装载外泌体。

A: loading of exosomes via transfection of the donor cell; B: loading of exosomes after their isolation.

图2 外泌体载药示意图

Fig.2 Schematic diagram of extracellular vesicle loading strategies

治疗效果, 说明外泌体可以将所携带的药物高效地运送到靶细胞发挥作用[45]。同时, 也有研究显示, 用质粒转染使HEK293T细胞来源的外泌体表面表达IL-3, 用该外泌体装载*Bcr-Abl*特异性siRNA后能够靶向性抑制*Bcr-Abl*慢性髓性白血病细胞的生长[46]。

外泌体除了可以包载小分子RNA外, 还可以包载小分子化疗药。利用EL-4细胞衍生的外泌体包载姜黄素, 能够显著提高姜黄素的循环时间和靶向性[47]。提取未成熟的DCs来源的外泌体, 以电转的方式装载化疗药阿霉素, 靶向治疗乳腺癌。研究发现, 外泌体装载的阿霉素可以递送至小鼠实体瘤中, 这为疏水性化学药物的递送提供了新途径[48], 而且可以显著降低患者的心脏损伤[49]。此外, 有研究显示紫杉醇和外泌体联合治疗肺癌也具有良好的效果[50]。

神经系统性肿瘤由于血脑屏障的存在, 导致放化疗效果不佳, 预后差等问题。Zhuang等[51]和Haney等[52]分别使用包载姜黄素和过氧化氢酶的外泌体, 通过鼻腔给药的方式在小鼠模型中证明了神经保护作用。最近的报道表明, 外泌体具有穿过BBB的内在机制[53], 研究者使用了具有完整BBB的人脑胶质瘤细胞的原位异种移植小鼠模型, 证实所有类型肿瘤细胞来源的外泌体均可穿过完整的BBB, 并可以在外周血中检测得到。因此, 利用外泌体作为药物载体携载化疗药, 有望成为有效的脑部肿瘤治疗方法。

4 总结与展望

外泌体是由细胞主动释放的纳米级膜囊泡, 尽管最初被认为是细胞产生的垃圾, 但随着研究的深入, 现在外泌体被认为是细胞间通讯的使者, 疾病诊断和治疗的重要载体, 特别是在肿瘤的诊断与治疗方面展现出得天独厚的优势。近几年来, 外泌体在肿瘤诊治领域发展迅速, 然而要应用于临床仍然有许多问题需要解决(表2)。首先, 到目前为止, 外泌体还不能规模化生产, 实验研究中所获得的外泌体远远不能满足临床应用。虽然有报道表明肿瘤细胞可以分泌较多的外泌体, 但肿瘤来源的外泌体可能存在促进肿瘤侵袭、迁移和增殖等安全隐患。Jang等[54]通过挤压法将单核细胞和巨噬细胞制成了类似外泌体的纳米囊泡, 这些囊泡对肿瘤细胞的作用与外泌体类似, 但是它们的产量却是外泌体的100倍。之后, 一种离心悬浮细胞制备类似外泌体纳米囊泡的装置

表2 外泌体在临床应用存在的问题及可能的解决方法

Table 2 Problems of exosomes in the clinical application and possible solutions

存在的问题 Problems	可能的解决方法 Possible solutions	参考文献 References
Low yield	Bioinspired exosome-mimetic nanovesicle or developing methods for mass production of EVs from cell culture	[55]
High separation and purification cost complicated operation	Developing simple and efficient extraction kit	[56]
Heterogeneity and quality control standards are difficult to unify	Establish standardized cell culture mode and strict quality control monitoring	[57]
Difficult to achieve active targeted drug delivery	Exosomes are surface-bound with specific ligands and specifically bind to the corresponding receptors of the target cells to achieve targeted administration	[58]
As a biomarker, the content of exosomes is less	The National Institutes of Health is developing a reference database for extracellular RNA as biomarkers or develop more sensitive instrumentation for single EV profiling	[11]

应运而生, 这种装置制备的纳米囊泡产量比外泌体高250倍[5]。因此, 利用仿生外泌体有望解决这一问题, 但它仍处于初步研究阶段。其次, 外泌体的提取有多种方法, 每种方法各有优缺点, 目前仍没有一种方法能做到兼顾外泌体的含量、纯度及生物活性。试剂盒提取法是近几年来新发展的一种方法, 不需要特殊设备, 且提取效率和纯化效果相对较高, 因而逐渐取代超速离心法并推广开来。

外泌体作为载体可以携带多种用于肿瘤诊断和治疗的生物大分子和药物分子, 但是如何实现高效的药物负载、提高靶向性以及外泌体在复杂肿瘤微环境中发挥作用的机制等方面仍需要深入研究, 而这些问题的解决将会给肿瘤诊治注入新的思想。

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