外泌体: 精准肿瘤学的明星

高弈航^{1,2}, 黄国翔², 唐玉国², 黄映辉^{1*}, 高山^{2*}

1. 北京工业大学生命科学与生物工程学院, 北京 100124;

2. 中国科学院生物医学检验技术重点实验室, 中国科学院苏州生物医学工程技术研究所, 苏州 215163

* 联系人, E-mail: yhuang@bjut.edu.cn; gaos@sibet.ac.cn

2018-03-06 收稿, 2018-03-29 修回, 2018-04-02 接受, 2018-05-11 网络版发表

国家重点研发计划(2016YFC1302100)、国家自然科学基金(81472827, 81773023)、中国科学院“百人计划”和中国科学院前沿科学重点研究项目(QYZDB-SSW-SMC038)资助

摘要 外泌体是指由细胞分泌具有生物活性的囊泡, 它直径一般为30~150 nm, 具有脂质双层膜。外泌体包含了分泌细胞的分子, 如酯类、蛋白质和核酸等。外泌体在生命体内承担着传递生物分子以及帮助细胞之间信息交流的角色。本文介绍了外泌体的定义、组成以及受体细胞内吞外泌体的方式, 探讨了外泌体在癌症液体活检以及在肿瘤发生、发展、转移和逃避免疫监视中的作用。系统地总结了外泌体作为肿瘤发生、发展以及恶性程度的生物标志物的研究进展, 并进一步探讨了外泌体在肿瘤治疗中作为治疗靶点以及载体所起到的作用和意义。最后对外泌体在临床中的潜在应用进行了展望, 为今后肿瘤诊断治疗提供新的思路。

关键词 外泌体, 肿瘤, 生物标志物, 诊断治疗, 精准医学

1983年, Pan等人^[1]在绵羊网织红细胞体外成熟的过程中, 用异硫氰酸荧光素或者125I标记的转铁蛋白受体的抗体, 对转铁蛋白受体进行追踪, 发现细胞膜的转铁蛋白受体在与一种包含多肽的囊泡结合后, 囊泡与细胞膜分离, 分泌到上清, 这是人类首次观测到游离于细胞外存在的囊泡。1987年, 他们进一步发现从绵羊网织红细胞分离的囊泡中含有在网织红细胞成熟过程中丢失的一些成分, 比如乙酰胆碱酯酶、葡萄糖转运蛋白、核苷转运蛋白和转铁蛋白受体等。同时, 他们发现这些囊泡膜具有网织红细胞细胞膜的特征, 比如也有极高的鞘磷脂含量, 显示了其区别于细胞或血小板等其他组分的独特性。他们将其命名为外泌体(exosome)^[2]。

在过去的几年中, 许多研究发现, 外泌体通过携带蛋白质及核酸等成分在细胞间传递信息并发挥功能^[3-5]。此外, 外泌体在肿瘤、神经退行性疾病、传染病以及新陈代谢相关疾病中也有作用^[6]。不同细胞分

泌的外泌体对疾病发生和发展有不同的影响。一些外泌体可促进疾病发生和发展, 而一些外泌体则抑制疾病发生和发展^[6]。已有研究证实, 外泌体所携带的相关物质不仅可作为疾病诊断的生物标志物, 还可以作为治疗靶点^[3]。本文总结了近年来以外泌体作为肿瘤诊断生物标志物和治疗靶点的最新研究进展, 为肿瘤的精准确诊和治疗提供新的方向和思路。

1 外泌体与肿瘤

1.1 外泌体的定义及研究方法

外泌体特指细胞分泌的腔内囊泡(intraluminal vesicles, ILVs), 直径约为30~150 nm, 具有“杯状”或“碟状”形态, 外侧含有脂质双分子层^[7]。外泌体是在细胞内由多囊泡胞内体(multivesicular bodies, MVBs)向外出芽形成, 通过MVBs与细胞膜融合, 由细胞主动分泌释放到细胞外。它被认为是细胞间分子交换

引用格式: 高弈航, 黄国翔, 唐玉国, 等. 外泌体: 精准肿瘤学的明星. 科学通报, 2018, 63: 2361-2368

Gao Y H, Huang K S, Tang Y G, et al. Exosome: A rising star in the era of precision oncology (in Chinese). Chin Sci Bull, 2018, 63: 2361-2368, doi: 10.1360/N972018-00118

和信息传递的生物纳米颗粒^[8-10].

外泌体主要可以通过透射电子显微镜(transmission electron microscopy, TEM)来观察,并测量其直径^[11]. 纳米粒子跟踪分析(nanoparticle tracking analysis, NTA)也能够测量外泌体的直径. 流式细胞技术(flow cytometry, FACS)可以分离外泌体并分析其纯度^[12]. 外泌体存在广泛但含量较低,分离和纯化外泌体是研究和应用的基础. 目前,有多种分离外泌体的方法,较为常用的是结合过滤步骤的超速离心法^[13]和超滤法^[12].

1.2 外泌体的生物学特点

外泌体广泛存在于生物体体液中,包括血液、唾液和尿液等,能被多种细胞分泌,对生物体具有重要的生物学意义. 早期人们认为外泌体的作用只是将代谢垃圾从细胞内运出细胞外. 直到1996年, Raposo等人^[14]发现外泌体具有呈递抗原,并刺激T细胞增殖的作用. 由此,人们逐渐发现外泌体具有一定的生理功能^[4,15],并开始受到人们的关注.

外泌体含有蛋白质、脂质、DNA以及RNA等生物分子^[16]. 不同细胞分泌的外泌体具有共同的生物分子,如TSG101^[12], HSP70^[17], CD9^[18], CD63^[19], FASN以及VCP^[20]等蛋白. 这些共同的蛋白分子可作为评价外泌体质量和纯度的生物标志物. 外泌体也具有来自母体细胞所特有的生物分子,例如在卵巢癌中,相对于低转移细胞系,高转移细胞系所分泌的外泌体中let-7 miRNA家族要丰富很多. 而miR-200家族成员则只在低转移细胞分泌的外泌体中存在^[21].

外泌体被细胞释放出来后随体液(如血清、唾液及脑脊髓液)流动到达受体细胞. 目前认为,外泌体进入受体细胞有3种模式(图1): (1) 外泌体直接与细胞膜融合; (2) 外泌体被细胞膜表面的受体识别,从而进入细胞; (3) 外泌体被受体细胞内吞进入细胞^[22]. 进入受体细胞后,外泌体通过向其释放内容物而发挥作用.

1.3 外泌体与肿瘤的关系

外泌体已被证实参与肿瘤发生、发展、转移、恶化和免疫逃逸过程中起到很重要的作用^[23,24]. 例如胶质瘤细胞分泌的外泌体可以促进其自身的增殖^[25]. Gu等人^[26]发现,间充质干细胞分泌的外泌体可以促进胃癌细胞的生长. 这些结果表明,外泌体可以促进

图1 (网络版彩色)外泌体进入受体细胞的3种模式

Figure 1 (Color online) Three models of the exosomes entering cells

肿瘤发生和发展. 进一步发现外泌体也能促进肿瘤细胞转移. Yu等人^[27]发现,乳腺癌分泌的外泌体可以促进肿瘤血管生成、肿瘤生长和扩散. 此外,乳腺癌细胞分泌的外泌体也可以促进周围正常乳腺细胞的增殖并抑制它们的凋亡. 在黑色素瘤中,其分泌的外泌体接触到淋巴结,刺激淋巴结产生相应的分子信号,影响黑色素瘤细胞重新聚集、细胞外基质沉积和淋巴结内的血管再生,促进了黑色素瘤的转移^[23]. 外泌体也有可能促进癌症病人出现并发症导致恶化. 例如Javeed等人^[28]发现,胰腺癌病人经常会患有糖尿病. 他们通过对比正常组织和癌组织发现,在癌组织分泌的外泌体中、肾上腺髓质素含量明显高于正常组织. 这种外泌体被附近的 β 细胞吞噬后,阻止了胰岛素分泌,因此导致糖尿病的发生.

最近,研究证实外泌体也参与免疫调节,会导致机体免疫抑制^[29,30]或激活^[24]. 例如黑色素瘤所分泌的外泌体膜上含有Fas配体分子(Fas ligand, FasL),可与T细胞的Fas受体相互作用从而介导T细胞凋亡以逃脱免疫监视^[30]. 相反, Wolfers等人^[24]发现,肿瘤分泌的外泌体膜上携带肿瘤细胞的抗原,被树突状细胞识别并呈递给T细胞,从而诱发免疫反应,促进免疫激活. 鉴于外泌体在肿瘤细胞中的作用,因此被视为肿瘤诊断和治疗的生物标志物及潜在的治疗靶点(图2).

2 外泌体对肿瘤诊断的意义

2.1 常用肿瘤液体活检方法

目前肿瘤诊断方法主要有影像、生化和病理学等

图2 精准肿瘤学中外泌体在肿瘤诊断与治疗中的应用

Figure 2 The applications of exosomes as diagnostic biomarkers and therapeutic targets in precision oncology

手段。然而, 这些方法存在肿瘤早期检测难、无法确诊癌症和容易引发癌细胞扩散等缺点^[31~33]。而液体活检具有微创和早期诊断肿瘤的优点, 因此越来越受到广泛的关注^[31]。

液体活检是一种用于临床肿瘤早期诊断和预后判定的检测手段, 具有安全、特异、便捷等特点。目前肿瘤液体活检主要有3种检测靶点: 循环肿瘤细胞(circulating tumor cells, CTCs)、循环肿瘤DNA/RNA(circulating tumor DNA/RNA, ctDNA/RNA)以及肿瘤细胞分泌的外泌体^[31]。这3种检测靶点对肿瘤病人的伤害较小, 而且通过它们可快速获得肿瘤的实时动态信息, 被认为是精准医学和个性化治疗的优选工具^[31]。

2.2 外泌体作为液体活检检测靶点的优点

在3种液体活检的检测靶点中, CTC的数量及其恶化程度对癌症检测具有不可低估的意义^[34]。然而, 它会出现随机性和不确定性, 且其检测并不能反映肿瘤的病理状态, 对肿瘤远端转移也不是一个可靠的标准^[31]。而ctDNA/RNA由于半衰期较短, 可以监测病人当时情况。但由于检测与肿瘤细胞类型、疾病进程、治疗手段等有关, 含量存在波动, 具有准确度较低、敏感性差的缺点^[31]。

相比于CTCs和ctDNA/RNA, 外泌体作为诊断手段则具有更加灵敏的优点, 是目前被看好的作为液体活检的检测靶点之一。它几乎存在于人体所有的体液里, 是液体活检的重要组成部分。它主要具有以

下3个优点: (1) 在体液中含量多于CTC, 每毫升血液超过10个, 比CTC更加灵敏^[35]; (2) 具有脂质双分子层结构, 能够保护内部的核酸分子免于被降解^[36]; (3) 由于体积较小, 通透能力较强, 较为容易进入体液从而被检测到^[37]。

2.3 外泌体成分中的癌症诊断生物标志物

研究已经证实, 包含癌细胞信息的外泌体被分泌到细胞外环境中^[38,39], 可以被利用作为乳腺癌^[22]、肺癌^[5,40]、卵巢癌^[41]等癌症早期诊断生物标志物。而这些可被作为生物标志物的生物分子主要分为两大类: 一类是外泌体中所包含的蛋白质分子, 而另一类则是外泌体所包含的核酸分子。

外泌体中的蛋白质主要包含来源于细胞膜蛋白和细胞质蛋白。这些蛋白的含量可部分反应癌细胞的状态, 具有作为衡量细胞生理状态生物标志物的潜力。在非小细胞肺癌(non-small cell carcinoma, NSCLC)病人体内癌细胞分泌的外泌体中, 含有食管鳞状上皮癌抗原1(New York esophageal squamous cell carcinoma 1, NY-ESO-1), 人表皮生长因子受体(epidermal growth factor receptor, EGFR), 人胎盘碱性磷酸酶(placental alkaline phosphatase, PLAP), 上皮细胞黏附分子(epithelial cell adhesion molecule, EpCam)以及凋亡连接基因2相互作用蛋白X(apoptosis-linked gene 2-interacting protein X, Alix), 这些蛋白的浓度与NSCLC病人低氧血症呈正相关^[42], 如果外泌体中富含NY-ESO-1则预示NSCLC病人的生存率比较低^[42]。Wong等人^[43]通过比较75个转移性胰腺癌病人在治疗前后糖类抗原19-9(carbohydrate antigen 19-9, CA19-9)的水平, 发现CA19-9低表达水平预示着病人较高的总生存率。Melo等人^[18]通过质谱分析鉴定了一种在胰腺癌病人的癌细胞外泌体中特有的蛋白多糖, 称为磷脂酰肌醇聚糖-1(glypican-1, GPC-1), 其表达水平与胰腺癌病人的肿瘤大小及肿瘤切除患者术后的生存密切相关, 是一个潜在的胰腺癌早期诊断生物标志物。Logozzi等人^[44]通过评估98个黑色素瘤患者和58个正常人血清中外泌体CD63和Caveolin-1分子的表达水平, 发现这两个蛋白在黑色素瘤患者的表达水平明显高于健康人, 暗示它们具有成为诊断生物标志物的潜力。除此之外, Skog及其同事^[25]在来自25个神经胶质母细胞瘤患者中的7个人的外泌体里分离到神经胶质母细胞瘤特异性表达的EGFRvIII(epi-

dermal growth factor receptor variant III)蛋白, 因此认为该分子可作为诊断神经胶质瘤的蛋白标志物。

外泌体中的核酸是另一种能够作为生物标志物的分子。目前, 已有多篇文献报道外泌体所包含的miRNA是理想的癌症早期诊断的生物标志物。Joyce等人^[32]通过分离50个乳腺癌患者及12个正常人外泌体样本, 发现miR-101, miR-372以及miR-373在乳腺癌患者外泌体中表达量均高于正常人。其中miR-373特定只在三阴性乳腺癌病人中特异性的高表达。同时, miR-373在雌/孕激素受体突变呈阴性的乳腺癌所分泌的外泌体中表达量显著高于受体突变呈阳性的患者, 也预示着其可以作为检测三阴性乳腺癌的生物标志物。Tanaka等人^[45]发现, 在食管鳞状细胞癌病人的血清中, 外泌体里的miR-21含量显著性升高, 且与肿瘤类型、肿瘤转移程度、淋巴侵袭程度和炎症转移的情况呈正相关。进一步Thind等人^[46]还发现, miR-21在肺癌、卵巢癌及胰腺癌中同样是判断癌症发生与否的生物标志物。Fang等人^[47]发现, 转移到肺部的肝癌细胞所分泌的外泌体中富含miR-1247-3p, 它能够通过降低 β -1,4-半乳糖转移酶3 (β -1,4-galactosyl-transferase 3, B4GALT3)的表达量以激活 β 1-integrin-NF- κ B通路, 从而诱使正常成纤维细胞转变为肿瘤相关成纤维细胞, 并提高白细胞介素6 (IL-6)和白细胞介素8 (IL-8)的分泌量, 促进肿瘤细胞的干性, 启动上皮-间充质(epithelial-mesenchymal transition, EMT)转化, 从而产生对化学药物的抗性。同时, 外泌体中高表达miR-1247-3p也与肝癌在肺部的转移密切相关, 因此, miR-1247-3p可以作为肝癌细胞是否转移到肺部的生物标志物。Qu等人^[48]发现, 对舒尼替尼耐药的肾癌细胞分泌的外泌体高表达长链非编码RNA ARSR (lncRNA activated in RCC with sunitinib resistance, lncARSR)。lncARSR通过竞争性结合miR-34/miR-449促进肾癌细胞中AXL和c-MET的表达, 从而产生耐药。因此, 外泌体中lncARSR的表达量可以作为诊断肾癌细胞对舒尼替尼耐药的生物标志物。全基因组测序结果显示胰管腺癌分泌到血清中的外泌体包含胰管腺癌中的全部基因组序列。而且还包含着导致胰管腺癌发生的驱动突变。因此, 外泌体中的DNA也可以作为诊断癌症发生的生物标志物^[49-52]。

综上所述, 肿瘤细胞分泌的外泌体由于其中丰富的内含物可作为诊断肿瘤发生、发展、转移及其恶化程度的标志物。

3 外泌体可作为肿瘤治疗的靶点和工具

外泌体具有促进细胞重编程^[53], 参与肿瘤细胞免疫逃逸^[54], 影响细胞成纤维化等功能^[55], 同时可以被癌细胞内吞^[56], 因此, 改造后的外泌体是肿瘤治疗的重要靶点和工具。

Lv等人^[54]发现, 肝癌患者在使用药物后, 癌细胞分泌的外泌体中, 其热休克蛋白70 (heat shock proteins 70, HSP70)含量上升, 从而提高了肝癌细胞的免疫原性并诱导了NK细胞自然杀伤能力, 对患者的治疗大有益处。因此他们提出了将含有HSP70的外泌体制成疫苗治疗肝癌。Fonsato等人^[53]发现, 在CD29的介导下, 人正常肝脏干细胞分泌的外泌体可以迅速被肝癌细胞内吞, 而其中的一些高丰度miRNAs能够抑制癌细胞增殖并诱导其重编程。将这些外泌体加到成淋巴细胞瘤或脑胶质母细胞瘤中, 也展现出同样的抗肿瘤活性。由此, 他们认为, 肝脏干细胞分泌的外泌体具有对多种肿瘤的抗癌作用, 可以作为治疗肿瘤的工具进行进一步的研究。GTPase激酶KRAS发生突变是导致胰腺癌发生的一种常见的驱动突变。然而, 以其为治疗靶点面临着很大挑战^[55]。Kamerkar等人^[56]通过加工由成纤维状间充质细胞分泌的外泌体, 使其含有能够靶向KRAS G12D特异性突变的siRNA或者shRNA, 可以有效抑制小鼠胰腺癌并明显提高小鼠的总生存率。这些结果表明, 基于外泌体携带siRNAs, miRNAs和shRNAs可以作为癌症靶向治疗的策略。Nabet等人^[55]发现, 乳腺癌细胞会阻断间充质成纤维细胞NOTCH-MYC信号通路, 从而导致其释放的外泌体中RN7SL1上升, 促使乳腺癌生长, 转移和治疗耐受。因此, 通过靶向外泌体中的RN7SL1也是可作为治疗乳腺癌的潜在手段。

综上所述, siRNA, miRNA, 相关蛋白以及小分子化合物等可被装进外泌体, 具有抗肿瘤特性外泌体也可被递送到靶组织从而发挥治疗作用, 说明外泌体可作为肿瘤治疗的靶点和工具。

4 总结和展望

最近, 研究外泌体的文献激增, 这一现象客观反映了外泌体在生理和疾病所起的重要性以及外泌体在临床应用的潜在前景。然而, 现阶段的研究刚刚为我们揭开外泌体在肿瘤作用机制的面纱, 对外泌体在肿瘤发生、发展、转移、恶化以及免疫调控过程中

起到的作用和其中的分子机制亟待进一步的探索。同时,外泌体的分离纯化成本较高、操作复杂,这些因素也限制了其在临床大面积推广使用。本文系统总结了外泌体的特征以及作为肿瘤诊断标志物和治疗靶点的研究进展和潜在前景。

外泌体以“使者”的身份让细胞与细胞,细胞与周围环境间建立起了双向交流。外泌体通过将携带的供体细胞的成分释放到受体细胞中,从而对受体细胞产生影响。目前,外泌体在肿瘤诊断与治疗中的作用逐渐被挖掘出来。部分研究认为外泌体可以通过触发免疫反应抑制肿瘤发生,但同时大量研究表明外泌体具有促进肿瘤发生和发展的作用。已有研

究表明肿瘤细胞可分泌比正常细胞更多的外泌体,且随着肿瘤恶化程度加深,外泌体分泌越来越多,而这些外泌体也携带肿瘤细胞特有的一些分子,暗示肿瘤分泌的外泌体作为早期诊断标志物和治疗靶点的可能性。

目前,利用纳米技术使外泌体负载药物或者特定基因,从而实现肿瘤靶向治疗的技术也在研发中。另外,通过外界手段改善外泌体在体内的表达水平达到治疗癌症患者的目的也为肿瘤治疗提供了更加广阔的前景。未来关于外泌体的研究将不会仅局限于它们在肿瘤中的生理作用,还会寻找它们在肿瘤诊断和治疗中作用的新途径。

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Summary for “外泌体: 精准肿瘤学的明星”

Exosome: A rising star in the era of precision oncology

Yihang Gao^{1,2}, Kuohsiang Huang², Yuguo Tang², Yinghui Huang^{1*} & Shan Gao^{2*}

¹ College of Life Science and Bioengineering, Beijing University of Technology, Beijing 100124, China;

² Key Laboratory of Bio-medical Diagnostics, Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, China

* Corresponding authors, E-mail: yhuang@bjut.edu.cn; gaos@sibet.ac.cn

Exosomes are dish-shaped phospholipid bilayer-enclosed membranous vesicles with diameter range of 30–150 nm. Exosomes as stable vesicles contain various bioactive molecules derived from the cells of parental origin, including proteins, lipids, DNA fragments, mRNAs, miRNAs, lncRNAs etc. Exosomes are secreted by almost all kinds of cells. Here, we focus on the application of exosomes in precision oncology. Nowadays, accumulating evidence suggests that cancer cells release the excessive amounts of exosomes compared to normal cells. The communications through exosomes between cancer cells and cancer/normal cells could make the exchange of genetic information and reprogramming of the recipient cells. Besides, the tumor-derived exosomes contribute the establishment of pre-metastatic niche and distant tissue site for metastasis of cancer cells. That may leads to cancer initiation, progression, metastasis of cancer cells and escaping from immune cell surveillance. In this review, we summarize the definition, composition and the potential diagnostic/therapeutic applications of exosomes. We introduce that there are several kinds of proteins enriched in exosomes such as TSG101, HSP70, CD9 and so on, which could be as the biomarkers to monitor the qualification and purification of exosomes we purified, in which play a pivotal role in exosome research. We describe the ways of recipient cells uptaking exosomes into themselves and the methods of exosome purification. Furthermore, we focus on non-invasive liquid biopsies in oncology, relying on analyzing material including molecules as well as whole cells that are found in bodily fluids such as blood and urine. Liquid biopsies are now in quick development and have been being an intriguing topic. We discuss the potential role of circulating tumor cells, circulating tumor-derived DNA/RNAs, and exosomes in liquid biopsy of cancer. We discriminate their characteristics and focus on exosomes that are used in liquid biopsy. Because the biomolecules in exosomes are derived from their parental cells, these contents are resembled between with each other. On this basis, it is conformed that they can reflect the dynamic information for genetic or signaling alteration of their cells of origin. We also highlighted its advanced progression in cancer diagnosis and therapeutic targets. It has been assumed that their contents such as nucleotides or proteins are abnormally enriched in tumor-derived ones compared to the normal, such as glypican-1 in pancreatic cancer and CD63 in the melanoma, which have the potential to be diagnostic biomarkers in cancers. The advanced studies have shown the difference between exosomes derived from tumor cells and normal cells, which may mediate different signaling pathways of the recipient cells. These facts indicate that we could transform exosomes to make it a medium to mediate agents to the recipient cells or a tool for treatment. For example, exosomes can serve as specific delivery vehicles loaded siRNAs/shRNAs targeted to mRNAs, proteins or small molecules, as a tool of gene therapy. We also point out that we are still in early step of understanding the nature and extent of the involvement of exosomes in physiology and therapeutic targets. We highlight that these will shed light on the new strategy for cancer diagnosis and therapy. The advanced studies in exosomes we presented may make greater contribution to overcome a variety of cancer in the era of precision oncology.

exosomes, cancer, biomarker, therapeutic target, precision medicine

doi: 10.1360/N972018-00118