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·综 述·

外泌体在肿瘤生物治疗中的研究进展

Research progress of exosomes in tumor biotherapy

万双双 综述;蔡志坚 审阅(浙江大学 医学院 免疫学研究所,浙江 杭州 310058)

[摘 要] 外泌体是各种细胞分泌的具有脂质双分子层膜结构囊泡状物质,广泛分布于机体组织中,同时也存在于肿瘤的微环境中,其对肿瘤的发生发展具有重要作用。由于细胞来源不同,外泌体在肿瘤的产生和进展中可发挥正向或负向调节作用。虽然目前对于这种截然相反现象的产生机制知之甚少,但是将具有抗肿瘤作用的外泌体应用于肿瘤治疗已经取得较大进展。本文就外泌体在肿瘤生物治疗研究中的进展作一综述,为外泌体作为抗肿瘤治疗的潜在载体及策略提供新的思路。

[关键词] 外泌体;肿瘤;生物治疗
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细胞向胞外释放囊泡是一种普遍的生理现象。细胞膜可以通过向外出芽的方式释放囊泡状物质,这些囊泡的直径介于100~1 000 nm,包括微囊泡、外泌体(exosomes)和微颗粒。Exosomes的直径往往小于150 nm[1-2]。根据国际外泌体协会的建议,以往文献中所认为的"exosomes"往往为包含各种囊泡的混合物。因此,本文采用"外泌体"这一名词包括其他类型胞外囊泡的exosomes。早期研究[3-4]揭示,外泌体起源于细胞的胞吞作用,在哺乳动物网织红细胞转变成红细胞的成熟过程中起重要作用。外泌体广泛存在于体液中,比如血浆或血清、唾液、尿液、母乳、脑脊液和精液等[5-7]。此外,肿瘤微环境中也存在大量的外泌体[5],提示外泌体在肿瘤的进展和抗肿瘤方面具有特殊作用。本文主要论述了外泌体在机体肿瘤进展过程中的正、负向作用以及外泌体在肿瘤生物治疗中的应用范例。

1 外泌体概述

外泌体是一群由细胞分泌的具有脂质双分子层结构的小囊泡,可用超速离心方法获得。外泌体可包含细胞来源的特异性蛋白,如抗原提呈细胞(antigen presenting cell, APC)来源的外泌体富集抗原提呈相关分子和MHC-Ⅰ、MHC-Ⅱ类分子及共刺激分子[8]。肿瘤细胞来源的外泌体包含肿瘤抗原和一些免疫抑制性蛋白,如FasL、TRAIL和TGF-β[9]。肿瘤来源的外泌体还可携带黏附分子、基质金属蛋白酶以及肿瘤原发和转移组织特异性相关蛋白,从而在肿瘤的迁移和侵袭中起重要作用[10]。有研究[11-12]表明,在许多癌症中,如肺癌、乳腺癌和前列腺癌等,通过检测外泌体中特定的microRNA,可判断外泌体是否来源于肿瘤细胞,在肿瘤的早期诊断中发挥作用。也有报道[13]表明,肿瘤细胞及其分泌的外泌体的mRNA和microRNA信号之间有很强的相关性。

2 外泌体的抗肿瘤作用

文献[14]表明,外泌体具有重要的抗肿瘤效应。自然杀伤(natural killer, NK)细胞通过释放含有穿孔素和颗粒酶的外泌体抑制肿瘤生长。将肿瘤来源的外泌体经基因修饰表达促炎因子如IL-12诱导特异性抗肿瘤反应[15]。而对淋巴细胞进行热激也可促进其释放高水平的包含MHC分子和共刺激分子的外泌体,诱导更有效的抗肿瘤反应[16]。最近也有人[17]发现,肿瘤细胞来源的外泌体通过DC介导的免疫反应起到抑制肝癌动物模型和人类肿瘤生长的作用。由于外泌体直径比较小,可通过血脑屏障进入脑部血液循环系统,外泌体可传递相关抗肿瘤RNA和蛋白质对脑部原发性肿瘤产生治疗作用[18-19]。有研究[20]称,经特异性microRNA转染的骨髓间充质干细胞产生的外泌体具有抗肿瘤作用。此外,外泌体在泌尿系统恶性肿瘤中起到重要的免疫调节作用[21]。含有MHC-Ⅰ类分子和肿瘤多肽的DC来源外泌体在动物模型中能诱导CTL反应,抑

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[作者简介] 万双双(1989-),女,黑龙江省齐齐哈尔市人,硕士生,主要从事肿瘤生物治疗的研究,E-mail:552197507@qq.com
[通信作者] 蔡志坚(CAI Zhijian, corresponding author),E-mail:caizj@zju.edu.cn

制肿瘤的生长[22]。

3　外泌体的促肿瘤作用

外泌体可通过诱导 CTL 凋亡及抑制 NK 细胞增殖大大减少机体具有杀伤肿瘤细胞作用的效应细胞数量,促进肿瘤的进展[23]。有证据表明肿瘤细胞来源的外泌体通过诱导 $CD4^+$ T 细胞分化为调节性 T 细胞(regulatory T cell, Treg),抑制机体的抗肿瘤免疫反应,介导对肿瘤的免疫耐受。含有 CD39 和 CD73 的肿瘤来源外泌体通过水解 ATP 产生大量腺苷,抑制 T 细胞的增殖与活化[24],引起肿瘤免疫逃逸。而来源于黑素瘤的含 Fas 配体的外泌体可诱导 T 细胞凋亡[25],抑制机体的抗肿瘤免疫反应。此外,来源于卵巢癌腹水的外泌体通过下调 T 细胞激活信号成分 CD3-zeta 和 JAK 3 表达产生免疫抑制效应,导致肿瘤的进展[26]。近期有研究[27-28]表明,外泌体通过上调其携带的 microRNA-222 和 microRNA-29a促进乳腺癌细胞的抗药性。

外泌体既有抗肿瘤作用同时又能促进肿瘤,其主要原因可能是外泌体作为细胞来源的外排小体,不同于单纯的细胞因子或者药物,外泌体可同时含有大量具有抗肿瘤及促肿瘤作用的蛋白成分或遗传信息,而最终外泌体在体内对肿瘤表现何种效应,取决于其抗肿瘤及促肿瘤作用何种起主导性作用。

4　人工修饰外泌体的抗肿瘤作用

外泌体的主要功能是介导不同细胞间的信息交流,包括转移蛋白、DNA、RNA 和 microRNA 等[29]。将外泌体设计成包含特异性生物激活分子用于治疗癌症。也可通过对来源细胞的基因改造,如使外泌体携带蛋白胞嘧啶脱氨酶融合尿嘧啶磷酸核糖转移酶的 mRNA 和蛋白,与化疗药 5-氟尿嘧啶联合应用可使肿瘤明显减小[30]。经生物工程合成改造供体细胞表达血小板衍生生长因子受体穿膜结构域与 GE11 多肽融合蛋白,使其产生的外泌体能够有效地将具有抗肿瘤作用的 let-7a microRNA 靶向传递给表达 EGFR 的乳腺癌细胞[31],促进乳腺癌的治疗。Rab27 是小 GTP 酶家族成员,它对调节外泌体的分泌有重要作用,通过小干扰 RNA 抑制 Rab27a 在乳腺癌细胞中的表达,会使肿瘤减小且降低乳腺癌细胞的肺转移[32-33]。

5　外泌体应用于肿瘤诊治的临床研究

肿瘤细胞来源的外泌体通常含有很多肿瘤抗原信息,能激活包括 DC 在内的 APC,激活的 DC 可诱导 CTL 依赖的抗肿瘤反应。Escudier 等[34]在 2000—2002 年期间进行了相关的临床Ⅰ期试验,将 MAGE3 致敏的自体 DC 产生的外泌体接种到转移性黑素瘤患者体内,显示出一定的抗肿瘤治疗效果和安全性。将自体 DC 产生的外泌体荷载黑素瘤抗原肽,应用于非小细胞肺癌的治疗,可在一定程度上激活机体的抗肿瘤免疫反应[35]。此外,恶性胸腔积液来源的外泌体能将肿瘤抗原信息提呈给 DC,在体外有效的诱导肿瘤特异性 CTL 扩增[36]。自体腹水来源的外泌体为结直肠癌免疫治疗提供了新的思路。外泌体通过传递相关的过氧化氢酶等具有治疗性的蛋白治疗帕金森病以及神经胶质瘤[38]。外泌体作为标志物还可对西妥昔单抗的治疗进行监测,从而判断疗效[39]。

有临床试验[40-43]证实,外泌体可能成为进展期非小细胞肺癌和结直肠癌的预后判断指标。同时,外泌体在前列腺癌和胰腺癌的一些分子诊断方法中也具有良好的应用前景[44-46],如通过磷脂酰肌醇聚糖-1 识别肿瘤来源的外泌体检测早期胰腺癌[47]。此外,有学者[48]用蛋白质组学分析出外泌体介导的戊糖磷酸化途径在卵巢癌迁移中起重要作用,这对卵巢癌的诊断和治疗将会起关键作用。

6　结　语

外泌体在肿瘤的发生发展中起重要作用,将外泌体通过自体回输应用于肿瘤生物治疗已经显示出良好的治疗效果,而外泌体用于肿瘤治疗的机制尚未研究透彻,目前临床应用仍有很大局限性。随着对外泌体研究的进一步深入,更多愈加复杂亟待解决的问题将随之而来,例如:外泌体在人体正常条件或病理条件下发挥的免疫调节作用是否相同? 外泌体在肿瘤发生发展不同阶段发挥的免疫调节作用是否相同? 相信未来随着外泌体发挥免疫调节作用机制的面纱不断被揭开,利用外泌体进行肿瘤治疗的前景将更广阔。

[参考文献]

[1] TKACH M, THERY C. Communication by extracellular vesicles: where we are and where we need to go[J]. Cell, 2016, 164(6): 1226-1232. DOI: 10.1016/j.cell.2016.01.043.

[2] TRAMS E G, LAUTER C J, SALEM N, et al. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles[J]. Biochim Biophys Acta, 1981, 645(1): 63-70.

[3] HARDING C, HEUSER J, STAHL P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat re-

ticulocytes [J]. J Cell Biol, 1983, 97(2): 329-339.

[4] PAN B T, TENG K, WU C, et al. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes [J]. J Cell Biol, 1985, 101(3): 942-948.

[5] VLASSOV A V, MAGDALENO S, SETTERQUIST R, et al. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials [J]. Biochim Biophys Acta, 2012, 1820(7): 940-948. DOI: 10.1016/j.bbagen.2012.03.017.

[6] LASSER C, ALIKHANI V S, EKSTROM K, et al. Human saliva, plasma and breast milk exosomes contain RNA: uptake by macrophages [J/OL]. J Transl Med, 2011, 9: 9 [2016-03-05]. http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-9-9. DOI: 10.1186/1479-5876-9-9.

[7] STREET J M, BARRAN P E, MACKAY C L, et al. Identification and proteomic profiling of exosomes in human cerebrospinal fluid [J/OL]. J Transl Med, 2012, 10: 5 [2016-03-05]. http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-10-5. DOI: 10.1186/1479-5876-10-5.

[8] MIGNOT G, ROUX S, THERY C, et al. Prospects for exosomes in immunotherapy of cancer [J]. J Cell Mol Med, 2006, 10(2): 376-388. DOI: 10.1111/j.1582-4934.2006.tb00406.x.

[9] CLAYTON A, MASON M D. Exosomes in tumour immunity [J]. Curr Oncol, 2009, 16(3): 46-49.

[10] KRUGER S, ABD ELMAGEED Z Y, HAWKE D H, et al. Molecular characterization of exosome-like vesicles from breast cancer cells [J/OL]. BMC Cancer, 2014, 14: 44 [2016-03-05]. http://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-14-44. DOI: 10.1186/1471-2407-14-44.

[11] ROSELL R, WEI J, TARON M. Circulating microrna signatures of tumor-derived exosomes for early diagnosis of non-small-cell lung cancer [J]. Clin Lung Cancer, 2009, 10(1): 8-9. DOI: 10.3816/CLC.2009.n.001.

[12] ORCORAN C, FRIEL A M, DUFFY M J, et al. Intracellular and extracellular microRNAs in breast cancer [J]. Clin Chem, 2011, 57(1): 18-32. DOI: 10.1373/clinchem.2010.150730.

[13] XIAO D, OHLENDORF J, CHEN Y, et al. Identifying mRNA, microRNA and protein profiles of melanoma exosomes [J/OL]. PLoS One, 2012, 7(10): e46874 [2016-02-21]. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0046874. DOI: 10.1371/journal.pone.0046874.

[14] LUGINI L, CECCHETTI S, HUBER V, et al. Immune surveillance properties of human NK cell-derived exosomes [J]. J Immunol, 2012, 189(6): 2833-2842. DOI: 10.4049/jimmunol.1101988.

[15] ZHANG Y, LUO C L, HE B C, et al. Exosomes derived from IL-12-anchored renal cancer cells increase induction of specific antitumor response in vitro: a novel vaccine for renal cell carcinoma [J]. Int J Oncol, 2010, 36(1): 133-140. DOI: 10.3892/ijo_00000484.

[16] CHEN W, WANG J, SHAO C, et al. Efficient induction of antitumor T cell immunity by exosomes derived from heat-shocked lymphoma cells [J]. Eur J Immunol, 2006, 36(6): 1598-1607. DOI: 10.1002/eji.200535501.

[17] RAO Q, ZUO B, LU Z, et al. Tumor-derived exosomes elicit tumor suppression in murine hepatocellular carcinoma models and human in vitro [J]. Hepatology, 2016, [Epub ahead of print] [2016-02-21]. DOI: 10.1002/hep.28549.

[18] KATAKOWSKI M, CHOPP M. Exosomes as tools to suppress primary brain tumor [J]. Cell Mol Neurobiol, 2016, 36(3): 343-352. DOI: 10.1007/s10571-015-0280-9.

[19] D'ASTI E, CHENNAKRISHNAIAH S, LEE T H, et al. Extracellular vesicles in brain tumor progression [J]. Cell Mol Neurobiol, 2016, 36(3): 383-407. DOI: 10.1007/s10571-015-0296-1.

[20] JOHNSEN K B, GUDBERGSSON J M, SKOV M N, et al. A comprehensive overview of exosomes as drug delivery vehicles-endogenous nanocarriers for targeted cancer therapy [J]. Biochim Biophys Acta, 2014, 1846(1): 75-87. DOI: 10.1016/j.bbcan.2014.04.005.

[21] JUNKER K, HEINZELMANN J, BECKHAM C, et al. Extracellular vesicles and their role in urologic malignancies [J]. Eur Urol, 2016, [Epub ahead of print] [2016-02-21]. http://linkinghub.elsevier.com/retrieve/pii/S0302283816002244. DOI: 10.1016/j.eururo.2016.02.046.

[22] HAO S, BAI O, LI F, et al. Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity [J]. Immunology, 2007, 120(1): 90-102. DOI: 10.1111/j.1365-2567.2006.02483.x.

[23] WHITESIDE T L. Immune modulation of T-cell and NK (natural killer) cell activities by TEXs (tumour-derived exosomes) [J]. Biochem Soc Trans, 2013, 41(1): 245-251. DOI: 10.1042/BST20120265.

[24] CLAYTON A, AL-TAEI S, WEBBER J, et al. Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production [J]. J Immunol, 2011, 187(2): 676-683. DOI: 10.4049/jimmunol.1003884.

[25] ANDREOLA G, RIVOLTINI L, CASTELLI C, et al. Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles [J]. J Exp Med, 2002, 195(10): 1303-1316. DOI: 10.1084/jem.20011624.

[26] TAYLOR D D, GERCEL-TAYLOR C. Tumour-derived exosomes and their role in cancer-associated T-cell signalling defects [J]. Brit J Cancer, 2005, 92(2): 305-311. DOI: 10.1038/sj.bjc.6602316.

[27] ZHONG S L, CHEN X, WANG D D, et al. MicroRNA expression profiles of drug-resistance breast cancer cells and their exosomes [J/OL]. Oncotarget, 2016, [Epub ahead of print] [2016-02-21]. http://www.impactjournals.com/oncotarget/index.php?journal=oncotarget&page=article&op=view&path%5b%5d=7481. DOI: 10.18632/oncotarget.7481.

[28] YU S T, WEI Y F, XU Y Q, et al. Extracellular vesicles in breast cancer drug resistance and their clinical application [J]. Tumor

Biol, 2016, [Epub ahead of print] [2016-02-21]. http://link.springer.com/article/10.1007%2Fs13277-015-4683-5. DOI: 10.1007/s13277-015-4683-5.

[29] RECO K A, FRANZEN C A, FOREMAN K E, et al. PLK-1 silencing in bladder cancer by siRNA delivered with exosomes [J]. Urology, 2016, [Epub ahead of print] [2016-02-21]. http://www.goldjournal.net/article/S0090-4295(16)00110-2/abstract. DOI: 10.1016/j.urology.2016.01.028.

[30] MIZRAK A, BOLUKBASI M F, OZDENER G B, et al. Genetically engineered microvesicles carrying suicide mRNA/protein inhibit schwannoma tumor growth [J]. Mol Ther, 2013, 21(1): 101-108. DOI: 10.1038/mt.2012.161.

[31] OHNO S, TAKANASHI M, SUDO K, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells [J]. Mol Ther, 2013, 21(1): 185-191. DOI: 10.1038/mt.2012.180.

[32] HENDRIX A, DE WEVER O. Rab27 GTPases distribute extracellular nanomaps for invasive growth and metastasis: implications for prognosis and treatment [J]. Int J Mol Sci, 2013, 14(5): 9883-9892. DOI: 10.3390/ijms14059883.

[33] BOBRIE A, KRUMEICH S, REYAL F, et al. Rab27a supports exosome-dependent and -independent mechanisms that modify the tumor microenvironment and can promote tumor progression [J]. Cancer Res, 2012, 72(19): 4920-4930. DOI: 10.1158/0008-5472.CAN-12-0925.

[34] ESCUDIER B, DORVAL T, CHAPUT N, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase Ⅰ clinical trial [J/OL]. J Transl Med, 2005, 3(1): 10 [2016-02-21]. http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-3-10. DOI: 10.1186/1479-5876-3-10.

[35] MORSE M A, GARST J, OSADA T, et al. A phase Ⅰ study of dexosome immunotherapy in patients with advanced nonsmall cell lung cancer [J/OL]. J Transl Med, 2005, 3(1): 9 [2016-02-21]. http://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-3-9. DOI: 10.1186/1479-5876-3-9.

[36] ANDRE F, SCHARTZ N E, MOVASSAGH M, et al. Malignant effusions and immunogenic tumour-derived exosomes [J]. Lancet, 2002, 360(9329): 295-305. DOI: 10.1016/S0140-6736(02)09552-1.

[37] DAI S, WEI D, WU Z, et al. Phase Ⅰ clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer [J]. Mol Ther, 2008, 16(4): 782-790. DOI: 10.1038/mt.2008.1.

[38] HALL J, PRABHAKAR S, BALAJ L, et al. Delivery of therapeutic proteins via extracellular vesicles: review and potential treatments for Parkinson's disease, glioma, and schwannoma [J/OL]. Cell Mol Neurobiol, 2016, [Epub ahead of print] [2016-02-21]. http://link.springer.com/article/10.1007%2Fs10571-015-0309-0. DOI: 10.1007/s10571-015-0309-0.

[39] VAN DOMMELEN S M, VAN DER MEEL R, VAN SOLINGE W W, et al. Cetuximab treatment alters the content of extracellular vesicles released from tumor cells [J]. Nanomedicine (Lond), 2016, 11(8): 881-890. DOI: 10.2217/nnm-2015-0009.

[40] TAVERNA S, GIALLOMBARDO M, GIL-BAZO I, et al. Exosomes isolation and characterization in serum is feasible in non-small cell lung cancer patients: critical analysis of evidence and potential role in clinical practice [J/OL]. Oncotarget, 2016, [Epub ahead of print] [2016-02-21]. http://www.impactjournals.com/oncotarget/misc/linkedout.php?pii=7638. DOI: 10.18632/oncotarget.7638.

[41] WANG Y Z, YI J, CHEN X G, et al. The regulation of cancer cell migration by lung cancer cell-derived exosomes through TGF-β and IL-10 [J]. Oncol Lett, 2015, 11(2): 1527-1530. DOI: 10.3892/ol.2015.4044.

[42] TOVAR-CAMARGO O A, TODEN S, GOEL A. Exosomal microRNA biomarkers: emerging frontiers in colorectal and other human cancers [J]. Expert Rev Mol Diagn, 2016, 16(5):553-567. DOI: 10.1586/14737159.2016.1156535.

[43] MUNAGALA R, AQIL F, GUPTA R C. Exosomal miRNAs as biomarkers of recurrent lung cancer [J/OL]. Tumor Biol, 2016, [Epub ahead of print] [2016-02-21]. http://link.springer.com/article/10.1007%2Fs13277-016-4939-8. DOI: 10.1007/s13277-016-4939-8.

[44] PUGLIESE D, PALERMO G, TOTARO A, et al. Clinical, pathological and molecular prognostic factors in prostate cancer decision-making process [J]. Urologia, 2016, 82(1): 14-20. DOI: 10.5301/uro.5000166.

[45] LU L, RISCH H A. Exosomes: potential for early detection in pancreatic cancer [J/OL]. Future Oncol, 2016, [Epub ahead of print] [2016-02-21]. http://www.futuremedicine.com/doi/abs/10.2217/fon-2015-0005?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed&. DOI: 10.2217/fon-2015-0005.

[46] SHARMA A, KHATUN Z, SHIRAS A. Tumor exosomes: cellular postmen of cancer diagnosis and personalized therapy [J]. Nanomedicine (Lond), 2016, 11(4): 421-437. DOI: 10.2217/nnm.15.210.

[47] MELO S A, LUECKE L B, KAHLERT C, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer [J]. Nature, 2015, 523(7559): 177-182. DOI: 10.1038/nature14581.

[48] YI H, ZHENG X Q, SONG J R, et al. Exosomes mediated pentose phosphate pathway in ovarian cancer metastasis: a proteomics analysis [J]. Int J Clin Exp Pathol, 2015, 8(12): 15719-15728.

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