Exosomes作为生物信息载体在肿瘤中的研究进展

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摘要 外来体(exosomes, EXO)是由细胞内多泡体(multivesicular body, MVB)与细胞膜融合并释放到细胞外的纳米级膜性小囊泡, 广泛分布于生物体液中, 具有机体耐受特性及诱导归巢能力。外来体可携带蛋白、运送RNA, 在肿瘤细胞间的物质传递和信息转导中起到重要作用。近年来, 外来体逐渐成为恶性肿瘤研究领域的关注热点, 特别是基于外来体的靶向治疗, 成为引领肿瘤治疗的新导向。该文就外来体作为生物信息载体在肿瘤中的最新研究作一综述。

关键词 外来体; 载体; 肿瘤

Advance of Exosomes as the Carriers of Biological Information in Cancer

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Abstract Exosomes are nanometer sized membrane vesicles widely distributed in biological fluids, which are released in the extracellular milieu following the fusion of the extemal membrane of multivesicular body (MVB) with plasma membrane, with the characters of body tolerance and specific homing ability. Exosomes can carry proteins and RNAs and may be involved in intercellular substance transporting and signaling of tumor. In recent years, the role of exosomes in malignant tumor has gradually become hot spots, and especially targeted therapy based on exosomes has become a new orientation in the treatment of cancer. This review will discuss the latest advances of exosomes as the carriers of biological information in the treatment of cancer.

Keywords exosome; carrier; tumor

1981年, Trams等[1]在研究正常细胞和肿瘤细胞株剥落的小泡时提出“外来体(exosomes, EXO)”一词。1987年, Johnstone等[2]首次在网织红细胞成熟过程中观察到外来体, 成功分离纯化并命名为exosomes。随后, Blanc等[3]展开了关于网织红细胞来源的外来体的广泛研究。1996年, Raposo等[4]发现, 人B淋巴细胞内一些有膜结构的小囊泡具有抗原提呈能力, 其表面表达丰富的主要组织相容性复合体(major histocompatibility complex, MHC)II类分子, 可呈递抗原给T细胞使之活化。此后, 外来体研究引起了广泛关注。而肿瘤细胞来源的外来体表面富含肿瘤抗原, 可通过抗原提呈细胞(antigen-presenting cell, APC)呈递给细胞毒性T淋巴细胞(cytotoxic T lymphocyte, CTL), 使其具备肿瘤杀伤效应[5]。由此,

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外来体在肿瘤领域的作用引起广大学者兴趣。近期, 外来体作为新型的纳米载体, 参与细胞间RNAs和蛋白质等生物信息的传递, 广泛应用于肿瘤领域研究, 尤其在肿瘤基因治疗方面, 开启了纳米医学研究新领域。

1 EXO概述

EXO是细胞内多泡体膜经内吞逆出芽形成许多小囊泡, 并与细胞质膜发生融合时, 细胞以胞吐的形式分泌到细胞外微环境中的膜性囊泡。外来体有一个较窄的直径范围(30~100 nm), 多种细胞如树突状细胞、肿瘤细胞等都能分泌外来体[5-6]。外来体还广泛存在于血浆[7]和尿液[8]等各种体液中。

外来体的分子组成与来源有很大的相关性, 其主要由蛋白质、脂质和核酸组成。外来体的蛋白质组学研究发现, 外来体所含蛋白质主要有两类: 一类是在所有的外来体上均有分布的普通蛋白质, 主要包括: 跨膜转运和融合相关蛋白(G蛋白、膜联蛋白、脂筏结构蛋白flotillin)、跨膜蛋白tetraspannins(CD9、CD63、CD81、CD82)和热休克蛋白(Hsp70、Hsp90)等[9-10]。其中, CD9、CD63等常可作为外来体的分子标志物[11]。另一类是存在于特定外来体的特异蛋白质, 如树突状细胞[12]和B淋巴细胞[13]来源的外来体上富含MHC和共刺激分子CD80、CD86, 肿瘤细胞来源的外来体则含有大量肿瘤抗原。

除了蛋白质外, 外来体还含有丰富的脂类。外来体脂质成分与其来源细胞膜的脂质成分有所不同, 与它们的母细胞相比, 外来体富含磷脂酰丝氨酸、双饱和磷脂酰乙醇胺、双饱和磷脂酰胆碱、鞘磷脂、神经节苷脂GM3和胆固醇[14-16]。此外, 外来体内还含有RNA和DNA片段。2007年, Valadi等[17]首次通过芯片分析发现, 小鼠和人的肥大细胞系来源的外来体含有mRNAs和miRNAs, 并随外来体分泌到细胞外微环境中。

外来体通过直接或间接方式接触靶细胞, 与不同细胞交换膜蛋白, 进而发挥其作为载体的生物学作用。网织红细胞成熟过程中通过分泌外来体帮助排除无用的蛋白(如转铁蛋白受体), 促进网织红细胞的成熟[2,18]。Zitvogel等[19]发现, 负载了肿瘤抗原的DC细胞来源的外来体可诱导抗肿瘤免疫。同时, 新近研究显示, 小鼠肥大细胞来源的外来体富含miRNAs, 外来体将miRNAs传递到人肥大细胞, 可诱导鼠源蛋白CDC6、CX7A2和锌指蛋白271的从头合成[17]。

2 EXO纯化与修饰

2.1 分离与纯化

将生物体液或细胞培养上清按标准四步离心法分离制备外来体是大多数研究采用的纯化法。即4 °C下, 300 ×*g*离心10 min; 1 200 ×*g*离心30 min; 10 000 ×*g*离心30 min; 100 000 ×*g*超速离心60 min, 所获得的沉淀即为外来体[20-21]。随着技术的不断发展, Clayton等[13]利用免疫亲和性提取方法从抗原呈递细胞中提取外来体, 较四步离心法简便快捷, 更利于临床推广。值得关注的是, 近期Lai及其研究团队[22-23]使用高性能液相色谱法(high-performance liquid chromatography, HPLC)提取大小均匀的外来体, 应用HPLC提取的外来体纯度相对高, 但设备昂贵。因此, 有待进一步探寻理想和高效的外来体提取新方法。

2.2 供体细胞选择

目前, 多种类型的细胞已被用来作为肿瘤研究中获得外来体的生产工厂, 其中未成熟树突状细胞(immature dendritic cells, imDCs)以其独特的表面蛋白质组成成为当前研究外来体的热门供体细胞[24]。ImDCs来源的外来体上的CD9, 可促进其与靶细胞膜的融合, 提高药物的传递[25]。此外, imDCs来源的外来体缺乏免疫刺激T细胞的表面标记物, 如CD40、CD86、MHC-II等, 其免疫原性降低, 负载肿瘤抗原后能有效诱导机体的防御性应答反应[26]。同时, 非小细胞肺癌的临床试验证实, imDCs衍生的外来体具有较好的安全性[27]。然而, 目前该细胞衍生的外来体受产量的限制, 阻碍了其在临床上的应用。

在胶质瘤研究中采用骨髓间充质干细胞(mesenchymal stem cell, MSC)作为外来体的供体细胞[28-29]。骨髓间充质干细胞来源的外来体用于药物递送有其独特的优势——外来体产量可观, 提示该细胞可能是外来体临床适用的高效来源[30]。然而, 该细胞来源的外来体抗肿瘤作用仍存在争议, 该细胞作为外来体的供体细胞在恶性肿瘤治疗中的作用仍有待研究[31-32]。因此, 外来体的组织特异性靶向与高效来源结合, 可能是研究者所期望的理想外来体。

2.3 负载方法

外来体负载内容物可通过多种方法来实现。目前采用最多的负载方法是通过在供体细胞中过表达某种基因, 将其负载到外来体, 从而完成与肿瘤细胞

间的传递[20,29]。例如, 供体细胞转染miR-214, 从而使其分泌的外来体高表达miR-214; 同样, 抗miRNAs抗体也能以同样的方法转染到供体细胞, 进而表达于外来体[28,33]。

电穿孔因其临床参数的易控性, 是外来体负载内容物的一个良好选择。Alvarez-Erviti等[34]采用电穿孔方法成功将siRNAs负载到外来体, 进而递送到肿瘤细胞抑制特定基因的表达。此外, 有研究利用转染试剂使siRNAs加载到外来体[35-36]。虽然, 转染剂Lipofectamine 2000能有效负载siRNAs到外来体, 并将其递送到受体细胞降低基因的表达, 但外来体中自身携带的siRNAs可能干扰所负载的siRNAs产生的影响[35-36]。HiPerFect转染剂siRNAs的装载效率较电穿孔法低。此外, 研究发现, 利用孵育法亦可成功将内容物负载到外来体[37]。利用外来体表面蛋白质、脂类和治疗内容物之间的相互作用优势, 形成高效的胞内负载复合物, 是下一步研究所要解决的关键。

3 EXO负载多种生物信息在肿瘤中的研究

3.1 EXO负载siRNA

从基因治疗的观点来看, 外来体最重要的属性莫过于它们在细胞水平调节遗传物质的转移[38]。裸体基因siRNA在肿瘤治疗过程中存在种种障碍, siRNA易降解、不易穿透细胞膜和易引起免疫反应。天然的载体如外来体, 可能提供一个有效的传递策略。

近年来, 多项研究表明, 外来体作为天然来源的纳米级囊泡可传递外源性RNA(包括siRNAs和miRNAs), 特异性地靶向组织或细胞, 从而达到敲除目的基因、抑制小鼠肿瘤生长的目的[34,39]。Shtam等[36]的研究结果表明, 将siRNAs导入外来体可成功地借助外来体将siRNAs传递到效应靶细胞, 从而达到肿瘤基因治疗。研究者将腹水和HeLa细胞来源的外来体与siRNAs、脂质体的混合物室温孵育, 成功且高效地将外源性siRNAs负载到外来体, 负载了siRNAs的外来体分别与靶细胞HeLa、HT1080细胞共培养后, 共聚焦显微镜和流式细胞分析显示通过外来体的介导, siRNAs被高效地传递到靶细胞。更为重要的是, 该siRNAs的传递可沉默转录后基因, 诱导受体细胞的凋亡。

可见, 外来体可能成为一个有效传递siRNAs的平台; 同时, 这也可能成为潜在的肿瘤治疗策略。

3.2 EXO负载microRNA

近期研究发现, 大量的非编码RNA, 包括miRNAs和天然反义RNAs, 参与肿瘤的发展过程[40-41]。外来体自身可携带miRNAs, 因此在不同疾病模型的治疗中, 表现出良好的应用前景[17]。Chiba等[42]为了检测结直肠癌细胞来源的外来体能否将miRNAs传递给HepG2和A549细胞, 将结直肠癌细胞来源的外来体进行PKH67(绿色荧光染料)染色。随后与HepG2、A549共培养, 通过激光共聚焦显微镜观察到来源于结直肠癌细胞系的外来体可被HepG2、A549细胞所摄取, 且外来体所包含的miRNAs可在细胞间穿梭。

基于外来体作为细胞间通讯的媒介, 其与化疗耐药密切相关。越来越多的证据证实, 肿瘤来源的外来体在肿瘤治疗失败中扮演重要的角色。Chen等[43]发现, 外来体可介导miRNAs在细胞间传递实现耐药性的传播。从人类乳腺癌细胞MCF-7耐药亚系中提取外来体, 与母本细胞来源的外来体相比, 有441种miRNAs明显高表达。在随后的实验中, 他们将耐药亚系来源的外来体与母本细胞共同孵育, 定量PCR结果显示, 与耐药亚系来源的外来体共孵育的母本细胞miRNAs水平比对照组显著增高。更为重要的是, 耐药亚系来源的外来体共孵育的母本细胞出现凋亡减少和对化疗药物有较高水平的耐药性。另有研究发现, THP-1细胞来源的外来体可进入人HMEC-1细胞传递miR-150, 降低c-Myb的表达, 进而促进HMEC-1细胞的迁移性[44-45]。

3.3 EXO负载mRNA/蛋白质

在肿瘤治疗中, 将治疗基因传递到肿瘤细胞, 替换功能失调的基因, 可诱导免疫排斥反应或诱发肿瘤细胞的凋亡。Mizrak等[46]研究发现, 外来体可作为细胞来源的运载工具, 介导mRNA/蛋白质在细胞间传递, 应用于肿瘤的治疗。将HEK-293T细胞转染CD-UPRT脂质体, 从而获得富含CD-UPRT mRNA/蛋白质的外来体, 并于小鼠神经鞘瘤模型中瘤内注射该外来体, 在细胞质膜和蛋白质的相互作用下, 靶细胞可内吞或融合外来体, 释放外来体包裹的CD-UPRT mRNA/蛋白质到靶细胞。这些外来体与5-FC共同治疗小鼠神经鞘瘤, 可促进5-FC到5-FdUMP的转换[46-47], 显著诱导肿瘤细胞凋亡和肿瘤消退, 进一步突显出外来体包裹mRNA/蛋白质在恶性肿瘤治疗中的潜力。

另一方面, 在骨髓细胞定向分化为树突状细胞的过程中, 卵清蛋白、α-半乳糖神经酰胺与之共培养过夜, 提取的外来体富含该蛋白质和糖脂抗原。进一步的结果显示, 负载αGC-OVA的外来体可激活iNK细胞, 克服aGC在人体的无效能反应, 增强肿瘤特异性的适应性免疫应答, 促进肿瘤新型免疫治疗的发展[48]。

更为重要的是, Wang等[49]发现, 经CD40L修饰的肺癌细胞衍生的外来体, 亦携带丰富的CD40L, 与DC共培养后, DC表面表达的MHCII、CD80、CD8、CD40及分泌的IL-12均升高。动物模型试验显示, CD40L-EXO可诱导较强的抗肿瘤免疫反应。

目前, 多个外来体相关研究已进入临床试验阶段, 其中一项临床I期试验是提取经热处理的CEA^+肿瘤细胞系LS-174T的外来体, 该外来体富含CEA、HSP70和MHCI, 将其与CEA^+患者血清提取的单个核细胞共培养, 将产生的致敏DCs回输患者体内, 诱导强烈的CEA特异性的T细胞抗肿瘤作用。该试验结果显示, 60%患者产生了CEA特异性T细胞反应, 且耐受良好, 但是免疫刺激性影响有限, 有待进一步优化[50]。

3.4 EXO负载其他内容物

以外来体为基础的药物传递绝大多数探究的是干扰RNA的转移治疗, 研究发现, 其他类型的治疗内容物也可负载到外来体。

新近研究发现, 将imDCs来源外来体进行iRGD修饰及电穿孔技术负载多柔比星, 通过Lamp2b的共表达靶向αv整合素[51-52], 可实现对高表达αv整合素的人乳腺癌细胞系MDA-MB-231、MCF-7, 小鼠恶性黑色素瘤细胞系B16-F10和人肝细胞癌细胞系HepG2等的定向传递, 最终将多柔比星高效靶向传递至病灶[53-54]。众所周知, 姜黄素因稳定性差和生物利用度低, 阻碍了其在癌症和其他炎症性疾病的应用[55], 而外来体可提高天然药物姜黄素的稳定性。研究显示, 姜黄素与外来体共同孵育, 可成功将其负载到外来体。通过疏水作用, 姜黄素可自组装成外来体的脂质双分子层, 防止姜黄素的降解, 提高其稳定性和生物利用度[56]。

近期, Hood等[57]发现, 将外来体电穿孔时的PBS缓冲液替换成海藻糖缓冲液(trehalose pulse media, TPM), 可成功将较重的负载物(如超顺磁性氧化铁纳米颗粒)负载到外来体, 不仅实现半合成纳米颗粒在外来体的负载, 同时突显了外来体在磁共振成像诊断肿瘤中的潜力。

目前, 对外来体的研究大多围绕其正常生理和

表1 外来体作为载体在肿瘤中研究的相关文献

Table 1 Articles involving exosomes as delivery platforms in cancer research

外来体负载物 Exosome payload	外来体来源 Exosome source	治疗意义 Therapeutic potential	参考文献 Reference
siRNA	Mouse dendritic cells	Strong mRNA and protein knockdown of BACE1, a therapeutic target in Alzheimer's disease	[34]
siRNA	HeLa, HT1080	Exosome-delivered siRNAs were effective at causing post-transcriptional gene silencing	[36]
miRNA	HEK293	Exosomes can efficiently deliver miRNA to EGFR-expressing breast cancer cells	[39]
miRNA	HCT-15, SW480, WiDr	Exosomal RNAs can shuttle between cells	[42]
miRNA	MCF-7	The intercellular transfer of specific miRNAs may spread resistance by exosomes	[43]
miRNA	THP-1	Exosomes can deliver miR-150 to regulate target gene expression and to promote HMEC-1 cell migration	[44]
mRNA/protein	HEK-293T	Microvesicles can effectively deliver therapeutic mRNA/proteins to treatment of diseases	[46]
αGC-OVA	Mouse dendritic cells	Exosomes loaded with protein antigen and αGC will activate adaptive immunity in the absence of triggering iNKT-cell anergy	[48]
Doxorubicin	Mouse dendritic cells	Exosomes can deliver Dox specifically to tumor tissues, leading to inhibition of tumor growth without overt toxicity	[54]
5 nm superparamagnetic iron oxide nanoparticles (SPION5)	Mouse B16-F10 cells	Loading exosomes with SPION5 introduces the potential for MRI driven theranostic exosome investigations	[57]

细胞间作为miRNA、mRNA和蛋白载体的传递功能, 而外来体作为载体传递其他内容物, 例如化疗药, 研究甚少。因此, 探索外来体新型的负载内容物, 研究外来体负载内容物的适合条件将成为未来研究的一个新趋势。

4 前景与展望

外来体在肿瘤领域的研究取得了一定的进展, 然而仍面临巨大挑战。在细胞和分子治疗方面, 外来体是个不错的选择。外来体可介导基于免疫细胞类型的免疫系统的调节。虽然, 我们已经了解了关于外来体生物特性的大量信息, 但仍有许多工作要做, 以确保外来体在治疗方面的安全性和有效性。例如, 外来体成分的确定是大批量生产安全、有效的合成外来体的前提。此外, 因给药途径、剂量和成本等因素的变化, 外来体负载的设计原则仍需进一步改善。外来体凭借其独特的特性可以被用来创建一个以外来体为基础的递送系统, 且其优于人造载体。基于外来体在体液中的稳定性和其可修饰的表面蛋白, 外来体递送核酸物质到目标组织已经切实可行。期待在今后的研究中, 外来体能够有更加振奋人心的发现和更加广泛的用途。

参考文献 (References)

1 Trams EG, Lauter CJ, Salem N Jr, Heine U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. Biochim Biophys Acta 1981; 645(1): 63-70.

2 Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987; 262(19): 9412-20.

3 Blanc L, De Gassart A, Geminard C, Bette-Bobillo P, Vidal M. Exosome release by reticulocytes—an integral part of the red blood cell differentiation system. Blood Cells Mol Dis 2005; 35(1): 21-6.

4 Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, *et al*. B lymphocytes secrete antigen-presenting vesicles. J Exp Med 1996; 183(3): 1161-72.

5 Stucci S, Tucci M, Ascierto PA, Passarelli A, Mariaelena C, Madonna G, *et al*. Dendritic cell-derived exosomes (Dex) are potential biomarkers of response to Ipilimumab in metastatic melanoma. J Transl Med 2015; 13: 2083.

6 Wolfers J, Lozier A, Raposo G, Regnault A, Thery C, Masurier C, *et al*. Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nat Med 2001; 7(3): 297-303.

7 Rodriguez M, Silva J, Lopez-Alfonso A, Lopez-Muniz MB, Pena C, Dominguez G, *et al*. Different exosome cargo from plasma/bronchoalveolar lavage in non-small-cell lung cancer. Genes Chromosomes Cancer 2014; 5(9): 713-24.

8 Beckham CJ, Olsen J, Yin PN, Wu CH, Ting HJ, Hagen FK, *et al*. Bladder cancer exosomes contain EDIL-3/Del1 and facilitate cancer progression. J Urol 2014; 192(2): 583-92.

9 Franzen CA, Simms PE, van Huis AF, Foreman KE, Kuo PC, Gupta GN. Characterization of uptake and internalization of exosomes by bladder cancer cells. Biomed Res Int 2014; 2014: 619829.

10 Subra C, Grand D, Laulagnier K, Stella A, Lambeau G, Paillasse M, *et al*. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. J Lipid Res 2010; 51(8): 2105-20.

11 Lasser C, Eldh M, Lotvall J. Isolation and characterization of RNA-containing exosomes. J Vis Exp 2012; 59: e3037.

12 Wang Z, Ding L, Zheng XL, Wang HX, Yan HM. DC-derived exosomes induce osteogenic differentiation of mesenchymal stem cells. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2014; 22(3): 600-4.

13 Clayton A, Court J, Navabi H, Adams M, Mason MD, Hobot JA, *et al*. Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry. J Immunol Methods 2001; 247(1/2): 163-74.

14 Subra C, Laulagnier K, Perret B, Record M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. Biochimie 2007; 89(2): 205-12.

15 Vidal M, Sainte-Marie J, Philippot JR, Bienvenue A. Asymmetric distribution of phospholipids in the membrane of vesicles released during *in vitro* maturation of guinea pig reticulocytes: Evidence precluding a role for "aminophospholipid translocase". J Cell Physiol 1989; 140(3): 455-62.

16 Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Mobius W, Hoernschemeyer J, *et al*. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem 2003; 278(13): 10963-72.

17 Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9(6): 654-9.

18 Johnstone RM, Mathew A, Mason AB, Teng K. Exosome formation during maturation of mammalian and avian reticulocytes: Evidence that exosome release is a major route for externalization of obsolete membrane proteins. J Cell Physiol 1991; 147(1): 27-36.

19 Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, *et al*. Eradication of established murine tumors using a novel cell-free vaccine: Dendritic cell-derived exosomes. Nat Med 1998; 4(5): 594-600.

20 Escola JM, Kleijmeer MJ, Stoorvogel W, Griffith JM, Yoshie O, Geuze HJ. Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. J Biol Chem 1998; 273(32): 20121-7.

21 Lamparski HG, Metha-Damani A, Yao JY, Patel S, Hsu DH, Ruegg C, *et al*. Production and characterization of clinical grade exosomes derived from dendritic cells. J Immunol Methods 2002; 270(2): 211-26.

22 Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, *et al*. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res 2010; 4(3): 214-22.

23 Lai RC, Arslan F, Tan SS, Tan B, Choo A, Lee MM, *et al*. Derivation and characterization of human fetal MSCs: An alternative cell source for large-scale production of cardioprotective microparticles. J Mol Cell Cardiol 2010; 48(6): 1215-24.

24 Yin W, Ouyang S, Li Y, Xiao B, Yang H. Immature dendritic cell-derived exosomes: A promise subcellular vaccine for autoimmunity. Inflammation 2013; 36(1): 232-40.

25 van den Boorn JG, Schlee M, Coch C, Hartmann G. SiRNA delivery with exosome nanoparticles. Nat Biotechnol 2011; 29(4): 325-6.

26 Quah BJ, O'Neill HC. The immunogenicity of dendritic cell-derived exosomes. Blood Cells Mol Dis 2005; 35(2): 94-110.

27 Morse MA, Garst J, Osada T, Khan S, Hobeika A, Clay TM, *et al*. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med 2005; 3(1): 9.

28 Munoz JL, Bliss SA, Greco SJ, Ramkissoon SH, Ligon KL, Rameshwar P. Delivery of functional anti-miR-9 by mesenchymal stem cell-derived exosomes to glioblastoma multiforme cells conferred chemosensitivity. Mol Ther Nucleic Acids 2013; 2: e126.

29 Katakowski M, Buller B, Zheng X, Lu Y, Rogers T, Osobamiro O, *et al*. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. Cancer Lett 2013; 335(1): 201-4.

30 Chen TS, Arslan F, Yin Y, Tan SS, Lai RC, Choo AB, *et al*. Enabling a robust scalable manufacturing process for therapeutic exosomes through oncogenic immortalization of human ESC-derived MSCs. J Transl Med 2011; 9: 47.

31 Zhu W, Huang L, Li Y, Zhang X, Gu J, Yan Y, *et al*. Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth *in vivo*. Cancer Lett 2012; 315(1): 28-37.

32 Bruno S, Collino F, Deregibus MC, Grange C, Tetta C, Camussi G. Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth. Stem Cells Dev 2013; 22(5): 758-71.

33 Chen L, Charrier A, Zhou Y, Chen R, Yu B, Agarwal K, *et al*. Epigenetic regulation of connective tissue growth factor by MicroRNA-214 delivery in exosomes from mouse or human hepatic stellate cells. Hepatology 2014; 59(3): 1118-29.

34 Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 2011; 29(4): 341-5.

35 Wahlgren J, De LK, Brisslert M, Vaziri Sani F, Telemo E, Sunnerhagen P, *et al*. Plasma exosomes can deliver exogenous short interfering RNA to monocytes and lymphocytes. Nucleic Acids Res 2012; 40(17): e130.

36 Shtam TA, Kovalev RA, Varfolomeeva EY, Makarov EM, Kil YV, Filatov MV. Exosomes are natural carriers of exogenous siRNA to human cells *in vitro*. Cell Commun Signal 2013; 11: 88.

37 Jang SC, Kim OY, Yoon CM, Choi DS, Roh TY, Park J, *et al*. Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. ACS Nano 2013; 7(9): 7698-710.

38 Zomer A, Vendrig T, Hopmans ES, van Eijndhoven M, Middeldorp JM, Pegtel DM. Exosomes: Fit to deliver small RNA. Commun Integr Biol 2010; 3(5): 447-50.

39 Ohno S, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, *et al*. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. Mol Ther 2013; 21(1): 185-91.

40 Kohno K, Chiba M, Murata S, Pak S, Nagai K, Yamamoto M, *et al*. Identification of natural antisense transcripts involved in human colorectal cancer development. Int J Oncol 2010; 37(6): 1425-32.

41 Lin T, Dong W, Huang J, Pan Q, Fan X, Zhang C, *et al*. MicroRNA-143 as a tumor suppressor for bladder cancer. J Urol 2009; 181(3): 1372-80.

42 Chiba M, Kimura M, Asari S. Exosomes secreted from human colorectal cancer cell lines contain mRNAs, microRNAs and natural antisense RNAs, that can transfer into the human hepatoma HepG2 and lung cancer A549 cell lines. Oncol Rep 2012; 28(5): 1551-8.

43 Chen WX, Liu XM, Lv MM, Chen L, Zhao JH, Zhong SL, *et al*. Exosomes from drug-resistant breast cancer cells transmit chemoresistance by a horizontal transfer of microRNAs. PLoS One 2014; 9(4): e95240.

44 Zhang Y, Liu D, Chen X, Li J, Li L, Bian Z, *et al*. Secreted monocytic miR-150 enhances targeted endothelial cell migration. Mol Cell 2010; 39(1): 133-44.

45 Pan Q, Ramakrishnaiah V, Henry S, Fouraschen S, de Ruiter PE, Kwekkeboom J, *et al*. Hepatic cell-to-cell transmission of small silencing RNA can extend the therapeutic reach of RNA interference (RNAi). Gut 2012; 61(9): 1330-9.

46 Mizrak A, Bolukbasi MF, Ozdener GB, Brenner GJ, Madlener S, Erkan EP, *et al*. Genetically engineered microvesicles carrying suicide mRNA/protein inhibit schwannoma tumor growth. Mol Ther 2013; 21(1): 101-8.

47 Hamstra DA, Lee KC, Tychewicz JM, Schepkin VD, Moffat BA, Chen M, *et al*. The use of 19F spectroscopy and diffusion-weighted MRI to evaluate differences in gene-dependent enzyme prodrug therapies. Mol Ther 2004; 10(5): 916-28.

48 Gehrmann U, Hiltbrunner S, Georgoudaki AM, Karlsson MC, Naslund TI, Gabrielsson S. Synergistic induction of adaptive antitumor immunity by codelivery of antigen with alpha-galactosylceramide on exosomes. Cancer Res 2013; 73(13): 3865-76.

49 Wang J, Wang L, Lin Z, Tao L, Chen M. More efficient induction of antitumor T cell immunity by exosomes from CD40L gene-modified lung tumor cells. Mol Med Rep 2014; 9(1): 125-31.

50 Dai S, Wan T, Wang B, Zhou X, Xiu F, Chen T, *et al*. More efficient induction of HLA-A*0201-restricted and carcinoembryonic antigen (CEA)-specific CTL response by immunization with exosomes prepared from heat-stressed CEA-positive tumor cells. Clin Cancer Res 2005; 11(20): 7554-63.

51 Simhadri VR, Reiners KS, Hansen HP, Topolar D, Simhadri VL, Nohroudi K, *et al*. Dendritic cells release HLA-B-associated transcript-3 positive exosomes to regulate natural killer function. PLoS One 2008; 3(10): e3377.

52 Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, *et al*. Tissue-penetrating delivery of compounds and nanoparticles into tumors. Cancer Cell 2009; 16(6): 510-20.

53 Sloan EK, Pouliot N, Stanley KL, Chia J, Moseley JM, Hards DK, *et al*. Tumor-specific expression of alphavbeta3 integrin promotes spontaneous metastasis of breast cancer to bone. Breast Cancer Res 2006; 8(2): R20.

54 Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, *et al*. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials 2014; 35(7): 2383-90.

55 Deng ZB, Liu Y, Liu C, Xiang X, Wang J, Cheng Z, *et al*. Immature myeloid cells induced by a high-fat diet contribute to liver inflammation. Hepatology 2009; 50(5): 1412-20.

56 Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, *et al*. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther 2010; 18(9): 1606-14.

57 Hood JL, Scott MJ, Wickline SA. Maximizing exosome colloidal stability following electroporation. Anal Biochem 2014; 448: 41-9.