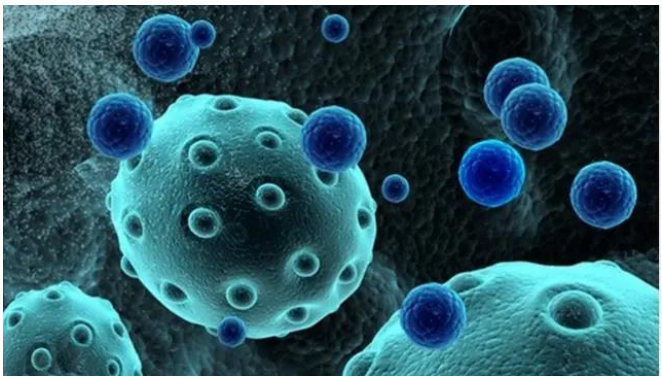
台灣幹細胞應用協會
TAIWAN STEM CELL APPLICATION NETWORK
科技新聞
NEWSLETTER
台內團字第1080060926號　促進幹細胞於人類健康與美麗上之福祉　總編輯 王振祥 博士

2021/4/27

外泌體不能亂來

王振祥 博士編撰



- 最近幹細胞外泌體在市場上非異常火紅，但定義不清，來源不明，可謂亂象一團。協會不得不撰此文以正視聽。
- 所有細胞都會分泌外泌體和其媒介誘導產生的各種旁泌因子，幹細胞會分泌好的外泌體和因子，但發炎細胞也會分泌助發炎的外泌體和因子，而且癌細胞也會分泌致癌和轉移的外泌體和因子。
- 癌症幹細胞和癌症的產生，以及癌症的轉移和此息息相關。或許癌症難以治癒和醫界沒有去處理腫瘤微環境中的外泌訊號因子有關。
- 要獲得有益的幹細胞外泌體和旁泌因子，幹細胞的來源和培養方法和環境是非常重要的。從有炎症或有癌症的源頭取得的幹細胞其實是非常危險的。培養方法和環境如果有造成變異的壓力也堪慮。
- 幹細胞培養會同時產生外泌體和旁泌因子來產生再生修復和免疫調節作用，執意將外泌體分離出來的意義何在值得討論，或許是藥廠想要將其專利壟斷為藥物。

旁分泌信號傳導 (Paracrine signaling) 對於維持細胞穩態是最重要的，並且在許多疾病的發作和傳播中也起著關鍵作用 [1-4]。 在過去的幾十年中，細胞分泌的可溶性因子，例如細胞因子和生長因子，被認為是細胞間旁分泌通訊的主要形式 [5-7]。 最近，細胞外囊泡（extracellular vesicles，EVs），特別是外泌體 (exosomes)，已被確定為旁分泌通訊的另一重要介體 [8-12]。 有關外泌體的出版物數量急劇增加，突顯了它們在科學研究中的重要性。

外泌體，大小在 40 至 150 nm 之間的納米囊泡，首先在培養的綿羊紅細胞的上清液中發現 [13-15]。然後，通過生物科學和技術的進步，發現這些納米囊泡被廣泛地生物分佈。目前，已經在幾乎所有類型的體液中發現了外泌體，包括唾液，牛奶，羊水，血清/血漿和尿液 [14，16-21]。外泌體可通過差速離心，密度梯度分離和市售試劑盒從各種液體中富集。並通過特定的生物標記物和粒徑鑑定[22-25]。

外泌體起源於通過細胞質膜的內吞作用 (endocytosis) 產生的內體 (endosomes) [26]。進一步處理後，外泌體通過膜融合 (membrane fusion) 釋放 [27]。它們被富含膽固醇，鞘磷脂和神經酰胺的脂質雙層包裹 [28，29]。外泌體的膜在一些四跨膜蛋白 (tetraspanins)（如 CD9，CD63 和 CD81）中也很豐富，可以用作鑑定外泌體的標誌物 [17、20、29-31]。外泌體的內部內容富含特殊的生物分子，功能蛋白和核酸，包括 microRNA（miRNA），信使 RNA（mRNA）甚至於 DNA [19，26，32，33]。

以前外泌體被認為是沒有用途的細胞代謝廢物，但當今人們已經認識到它們具有許多關鍵的細胞功能。釋放後，外泌體可以旁分泌的方式作用於親本細胞附近的特殊靶細胞，並且它們還可以進入生物體液（例如血液和尿液），被輸送到遠離分泌細胞的一些靶細胞，類似於傳統的內分泌作用過程 [19-21，33]。當外泌體被特定的靶細胞吸收時，外泌體的內含物，尤其是 miRNA，將介導許多生物學過程。外泌體中所含 miRNA 的潛在功能已經被進行許多深度的探索 [8，36-38]。詳細說來，最初的 miRNA 開始時是從親本細胞的基因組中轉錄而來，由 Drosha 加工成 pre-miRNA，然後轉運到細胞質中形成雙鏈成熟 miRNA。將成熟的 miRNA 整合到晚期內體中，然後釋放含 miRNA 的外泌體並被受體細胞捕獲。一股外泌體 miRNA 被整合到含有 miRNA 誘導的沉默複合物的 Argonaute 蛋白中，然後與靶 mRNA 轉錄物相

互作用，這通常導致相應基因表達的抑制 [26，32，34，35]。因此，外泌體中的某些生物分子可能被用作疾病診斷，預後甚至傷害狀況的生物標誌物，因為它們的水平或含量可能在某些疾病或傷害發生後發生變化 [32，36，39-42]。

在迄今為止報導的外泌體的功能中，主要研究了它們在癌症進展和免疫調節中的作用 [32，36，43]。 實際上，已發現癌細胞來源的外泌體以多種方式促進腫瘤形成和轉移，例如，通過將致癌因子轉移至正常細胞，重塑細胞外基質並介導免疫逃逸 [19、32、37、44， 45]。因此，已經探索了外泌體作為癌症診斷的潛在生物標誌物和癌症治療的特殊治療載體。 另外，通過在免疫細胞和其他細胞群之間轉移免疫調節細胞因子，miRNA 和/或其他介體，免疫細胞衍生的外泌體可起到促炎或抗炎劑的作用 [46-48]。

儘管外泌體功能已被廣泛探索，但調節組織修復和再生的潛力尚未引起人們的廣泛關注。然而，外泌體可能是許多當前細胞和組織工程策略的有前途的替代品 [49-51]。 支持這一觀點的最明顯證據來自於針對組織再生的間質幹細胞（MSC）移植的研究 [49，51-53]。 毫無疑問，通過一系列優雅的研究表明，MSC 主要通過旁分泌信號傳導，特別是通過它們產生的外泌體誘導細胞變化 [51-53]。 因此，我們可以合理地看到利用旁分泌因子（如外泌體）的無細胞療法，以促進組織修復和再生的願景，這將避免與直接幹細胞移植相關的風險，例如畸胎瘤，免疫排斥和 移植細胞的再生能力降低 [54-56]。

參考文獻

1. Muller E, Wang W, Qiao W, Bornhauser M, Zandstra PW, Werner C, et al. Distinguishing autocrine and paracrine signals in hematopoietic stem cell culture using a biofunctional microcavity platform. Scientific reports. 2016;6:31951.
2. Cho KI, Yoon D, Qiu S, Danziger Z, Grill WM, Wetsel WC, et al. Loss of Ranbp2 in motor neurons causes the disruption of nucleocytoplasmic and chemokine signaling and proteostasis of hnRNPH3 and Mmp28, and the development of amyotrophic lateral sclerosis (ALS)-like syndromes. Disease models & mechanisms. 2017.

3. Civitarese RA, Kapus A, McCulloch CA, Connelly KA. Role of integrins in mediating cardiac fibroblast-cardiomyocyte cross talk: a dynamic relationship in cardiac biology and pathophysiology. Basic research in cardiology. 2017;112:6.
4. Plotnikov EY, Silachev DN, Popkov VA, Zorova LD, Pevzner IB, Zorov SD, et al. Intercellular Signalling Cross-Talk: To Kill, To Heal and To Rejuvenate. Heart, lung & circulation. 2017.
5. Parascandolo A, Rappa F, Cappello F, Kim J, Cantu DA, Chen H, et al. Extracellular Superoxide Dismutase Expression in Papillary Thyroid Cancer Mesenchymal Stem/Stromal Cells Modulates Cancer Cell Growth and Migration. Scientific reports. 2017;7:41416.
6. Wang J, Sun B, Tian L, He X, Gao Q, Wu T, et al. Evaluation of the potential of rhTGF- beta3 encapsulated P(LLA-CL)/collagen nanofibers for tracheal cartilage regeneration using mesenchymal stems cells derived from Wharton's jelly of human umbilical cord. Materials science & engineering C, Materials for biological applications. 2017;70:637-45.
7. Blondiaux E, Pidial L, Autret G, Rahmi G, Balvay D, Audureau E, et al. Bone marrow-derived mesenchymal stem cell-loaded fibrin patches act as a reservoir of paracrine factors in chronic myocardial infarction. Journal of tissue engineering and regenerative medicine. 2017.
8. Zhang Y, Yu M, Dai M, Chen C, Tang Q, Jing W, et al. miR-450a-5p within rat adipose tissue exosome-like vesicles promotes adipogenic differentiation by targeting WISP2. Journal of cell science. 2017.
9. Collino F, Pomatto M, Bruno S, Lindoso RS, Tapparo M, Sicheng W, et al. Exosome and Microvesicle-Enriched Fractions Isolated from Mesenchymal Stem Cells by Gradient Separation Showed Different Molecular Signatures and Functions on Renal Tubular Epithelial Cells. Stem cell reviews. 2017.
10. Goloviznina NA, Verghese SC, Yoon YM, Taratula O, Marks DL, Kurre P. Mesenchymal Stromal Cell-derived Extracellular Vesicles Promote Myeloid-biased Multipotent Hematopoietic Progenitor Expansion via Toll-Like Receptor Engagement. The Journal of biological chemistry. 2016;291:24607-17.
11. Lee M, Ban JJ, Kim KY, Jeon GS, Im W, Sung JJ, et al. Adipose-derived stem cell exosomes alleviate pathology of amyotrophic lateral sclerosis in vitro. Biochemical and biophysical research communications. 2016;479:434-9.

12. Furuta T, Miyaki S, Ishitobi H, Ogura T, Kato Y, Kamei N, et al. Mesenchymal Stem Cell-Derived Exosomes Promote Fracture Healing in a Mouse Model. Stem cells translational medicine. 2016;5:1620-30.
13. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). The Journal of biological chemistry. 1987;262:9412-20.
14. Geminard C, Nault F, Johnstone RM, Vidal M. Characteristics of the interaction between Hsc70 and the transferrin receptor in exosomes released during reticulocyte maturation. The Journal of biological chemistry. 2001;276:9910-6.
15. Johnstone RM. Cleavage of the transferrin receptor by human granulocytes: differential proteolysis of the exosome-bound TFR. Journal of cellular physiology. 1996;168:333-45.
16. Katsiougiannis S, Chia D, Kim Y, Singh RP, Wong DT. Saliva exosomes from pancreatic tumor-bearing mice modulate NK cell phenotype and antitumor cytotoxicity. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2017;31:998-1010.
17. Hock A, Miyake H, Li B, Lee C, Ermini L, Koike Y, et al. Breast milk-derived exosomes promote intestinal epithelial cell growth. Journal of pediatric surgery. 2017.
18. Xiao GY, Cheng CC, Chiang YS, Cheng WT, Liu IH, Wu SC. Exosomal miR-10a derived from amniotic fluid stem cells preserves ovarian follicles after chemotherapy. Scientific reports. 2016;6:23120.
19. An M, Lohse I, Tan Z, Zhu J, Wu J, Kurapati H, et al. Quantitative proteomic analysis of serum exosomes from patients with locally advanced pancreatic cancer undergoing chemoradiotherapy. Journal of proteome research. 2017.
20. Domenyuk V, Zhong Z, Stark A, Xiao N, O'Neill HA, Wei X, et al. Plasma Exosome Profiling of Cancer Patients by a Next Generation Systems Biology Approach. Scientific reports. 2017;7:42741.
21. Sun H, Yao W, Tang Y, Zhuang W, Wu D, Huang S, et al. Urinary exosomes as a novel biomarker for evaluation of alpha-lipoic acid's protective effect in early diabetic nephropathy. Journal of clinical laboratory analysis. 2017.

22. Lee MJ, Park DH, Kang JH. Exosomes as the source of biomarkers of metabolic diseases. Annals of pediatric endocrinology & metabolism. 2016;21:119-25.
23. Mitchell P, Tollervey D. Finding the exosome. Advances in experimental medicine and biology. 2010;702:1-8.
24. Kastelowitz N, Yin H. Exosomes and microvesicles: identification and targeting by particle size and lipid chemical probes. Chembiochem : a European journal of chemical biology. 2014;15:923-8.
25. Wang D, Sun W. Urinary extracellular microvesicles: isolation methods and prospects for urinary proteome. Proteomics. 2014;14:1922-32.
26. Stahl PD, Barbieri MA. Multivesicular bodies and multivesicular endosomes: the "ins and outs" of endosomal traffic. Science's STKE : signal transduction knowledge environment. 2002;2002:pe32.
27. Montecalvo A, Larregina AT, Morelli AE. Methods of purification of CTL-derived exosomes. Methods Mol Biol. 2014;1186:87-102.
28. Rog T, Orlowski A, Llorente A, Skotland T, Sylvanne T, Kauhanen D, et al. Interdigitation of long-chain sphingomyelin induces coupling of membrane leaflets in a cholesterol dependent manner. Biochimica et biophysica acta. 2016;1858:281-8.
29. Simbari F, McCaskill J, Coakley G, Millar M, Maizels RM, Fabrias G, et al. Plasmalogen enrichment in exosomes secreted by a nematode parasite versus those derived from its mouse host: implications for exosome stability and biology. Journal of extracellular vesicles. 2016;5:30741.
30. Zhou Q, Rahimian A, Son K, Shin DS, Patel T, Revzin A. Development of an aptasensor for electrochemical detection of exosomes. Methods. 2016;97:88-93.
31. Oliveira-Rodriguez M, Lopez-Cobo S, Reyburn HT, Costa-Garcia A, Lopez-Martin S, Yanez-Mo M, et al. Development of a rapid lateral flow immunoassay test for detection of exosomes previously enriched from cell culture medium and body fluids. Journal of extracellular vesicles. 2016;5:31803.
32. Lai X, Wang M, McElyea SD, Sherman S, House M, Korc M. A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer. Cancer letters. 2017.

33. Mead B, Tomarev S. Bone Marrow-Derived Mesenchymal Stem Cells-Derived Exosomes Promote Survival of Retinal Ganglion Cells Through miRNA-Dependent Mechanisms. Stem cells translational medicine. 2017.
34. Beltrami C, Besnier M, Shantikumar S, Shearn AI, Rajakaruna C, Laftah A, et al. Human Pericardial Fluid Contains Exosomes Enriched with Cardiovascular-Expressed MicroRNAs and Promotes Therapeutic Angiogenesis. Molecular therapy : the journal of the American Society of Gene Therapy. 2017;25:679-93.
35. Yu X, Odenthal M, Fries JW. Exosomes as miRNA Carriers: Formation-Function-Future. International journal of molecular sciences. 2016;17.
36. Li J, Chen Y, Guo X, Zhou L, Jia Z, Peng Z, et al. GPC1 exosome and its regulatory miRNAs are specific markers for the detection and target therapy of colorectal cancer. Journal of cellular and molecular medicine. 2017.
37. Teng Y, Ren Y, Hu X, Mu J, Samykutty A, Zhuang X, et al. MVP-mediated exosomal sorting of miR-193a promotes colon cancer progression. Nature communications. 2017;8:14448.
38. Lim JH, Song MK, Cho Y, Kim W, Han SO, Ryu JC. Comparative analysis of microRNA and mRNA expression profiles in cells and exosomes under toluene exposure. Toxicology in vitro : an international journal published in association with BIBRA. 2017.
39. Khoontawad J, Pairojku C, Rucksaken R, Pinlaor P, Wongkham C, Yongvanit P, et al. Differential protein expression marks the transition from infection with Opisthorchis viverrini to cholangiocarcinoma. Molecular & cellular proteomics : MCP. 2017.
40. Cao XY, Lu JM, Zhao ZQ, Li MC, Lu T, An XS, et al. MicroRNA biomarkers of Parkinson's disease in serum exosome-like microvesicles. Neuroscience letters. 2017.
41. Cho YE, Kim SH, Lee BH, Baek MC. Circulating Plasma and Exosomal microRNAs as Indicators of Drug-Induced Organ Injury in Rodent Models. Biomolecules & therapeutics. 2017.
42. Panich T, Chancharoenthana W, Somparn P, Issara-Amphorn J, Hirankarn N, Leelahavanichkul A. Urinary exosomal activating transcriptional factor 3 as the early diagnostic biomarker for sepsis-induced acute kidney injury. BMC nephrology. 2017;18:10.

43. Rong L, Li R, Li S, Luo R. Immunosuppression of breast cancer cells mediated by transforming growth factor-beta in exosomes from cancer cells. Oncology letters. 2016;11:500-4.
44. Hu Y, Li D, Wu A, Qiu X, Di W, Huang L, et al. TWEAK-stimulated macrophages inhibit metastasis of epithelial ovarian cancer via exosomal shuttling of microRNA. Cancer letters. 2017.
45. Min H, Sun X, Yang X, Zhu H, Liu J, Wang Y, et al. Exosomes Derived from Irradiated Esophageal Carcinoma-Infiltrating T Cells Promote Metastasis by Inducing the Epithelial-Mesenchymal Transition in Esophageal Cancer Cells. Pathology oncology research : POR. 2017.
46. Momen-Heravi F, Bala S, Kodys K, Szabo G. Exosomes derived from alcohol-treated hepatocytes horizontally transfer liver specific miRNA-122 and sensitize monocytes to LPS. Scientific reports. 2015;5:9991.
47. Sukma Dewi I, Celik S, Karlsson A, Hollander Z, Lam K, McManus JW, et al. Exosomal miR-142-3p is increased during cardiac allograft rejection and augments vascular permeability through down-regulation of endothelial RAB11FIP2 expression. Cardiovascular research. 2017.
48. Ellwanger JH, Crovella S, Dos Reis EC, Pontillo A, Chies JA. Exosomes are possibly used as a tool of immune regulation during the dendritic cell-based immune therapy against HIV-I. Medical hypotheses. 2016;95:67-70.
49. Toh WS, Lai RC, Hui JH, Lim SK. MSC exosome as a cell-free MSC therapy for cartilage regeneration: Implications for osteoarthritis treatment. Seminars in cell & developmental biology. 2016.
50. Prathipati P, Nandi SS, Mishra PK. Stem Cell-Derived Exosomes, Autophagy, Extracellular Matrix Turnover, and miRNAs in Cardiac Regeneration during Stem Cell Therapy. Stem cell reviews. 2016.
51. Pashoutan Sarvar D, Shamsasenjan K, Akbarzadehlaleh P. Mesenchymal Stem Cell-Derived Exosomes: New Opportunity in Cell-Free Therapy. Advanced pharmaceutical bulletin. 2016;6:293-9.
52. Liu S, Liu D, Chen C, Hamamura K, Moshaverinia A, Yang R, et al. MSC Transplantation Improves Osteopenia via Epigenetic Regulation of Notch Signaling in Lupus. Cell metabolism. 2015;22:606-18.
53. Tan SS, Yin Y, Lee T, Lai RC, Yeo RW, Zhang B, et al. Therapeutic MSC exosomes are derived from lipid raft microdomains in the plasma membrane. Journal of extracellular vesicles. 2013;2.

54. Park S, Choi Y, Jung N, Yu Y, Ryu KH, Kim HS, et al. Myogenic differentiation potential of human tonsil-derived mesenchymal stem cells and their potential for use to promote skeletal muscle regeneration. International journal of molecular medicine. 2016;37:1209-20.
55. Iwai S, Sakonju I, Okano S, Teratani T, Kasahara N, Yokote S, et al. Impact of ex vivo administration of mesenchymal stem cells on the function of kidney grafts from cardiac death donors in rats. Transplantation proceedings. 2014;46:1578-84.
56. Merino A, Ripoll E, de Ramon L, Bolanos N, Goma M, Bestard O, et al. The Timing of Immunomodulation Induced by Mesenchymal Stromal Cells Determines the Outcome of the Graft in Experimental Renal Allotransplantation. Cell transplantation. 2017.