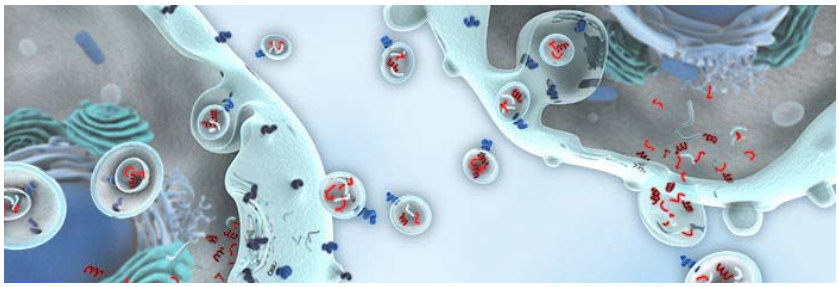
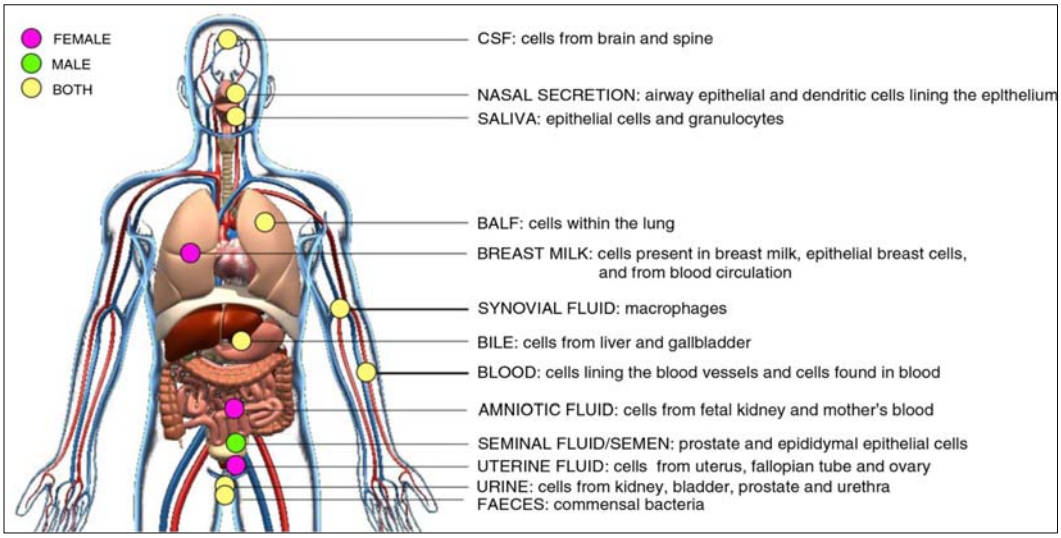


Microvescicole

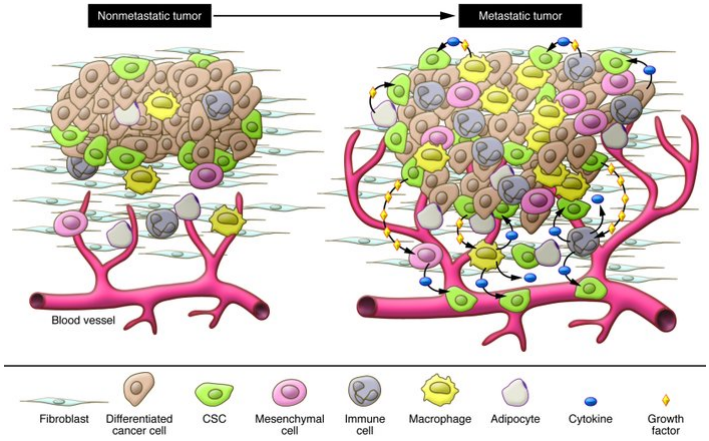
Seminari

http://biomarkerinsights.qiagen.com/category/liquid-biopsy/exosomes-microvesicles/

Vescicole extracellulari isolate dal fluidi corporei



Yáñez-Mó M. **Biological properties of extracellular vesicles and their physiological functions**. J Extracell Vesicles. 2015 May 14;4:27066..

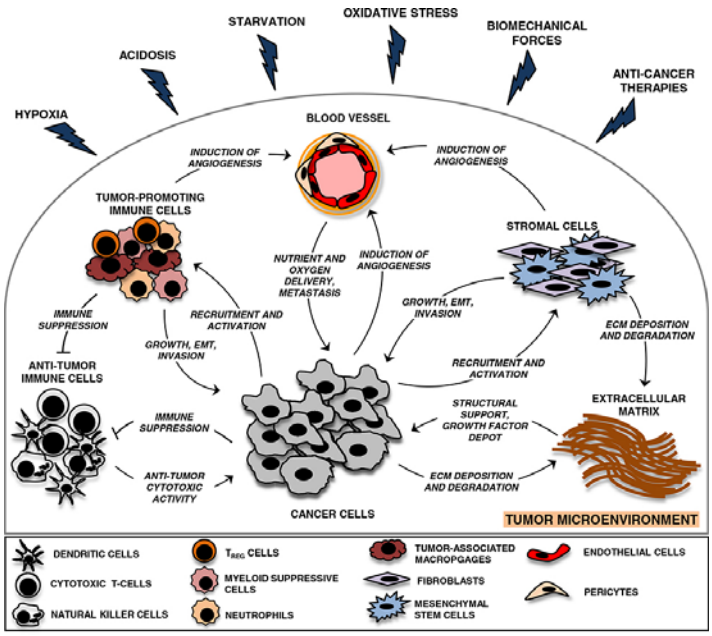


Microambiente tumorale

http://www.jci.org/articles/view/57099/figure/1

Considerable evidence has been gathered over the last 10 years showing that the tumor microenvironment (TME) is not simply a passive recipient of immune cells, but an active participant in the establishment of immunosuppressive conditions. It is now well documented that hypoxia, within the TME, affects the functions of immune effectors including natural killer (NK) cells by multiple overlapping mechanisms. Indeed, each cell in the TME, irrespective of its transformation status, has the capacity to adapt to the hostile TME and produce immune modulatory signals or mediators affecting the function of immune cells either directly or through the stimulation of other cells present in the tumor site. This observation has led to intense research efforts focused mainly on tumor-derived factors. Notably, it has become increasingly clear that tumor cells secrete a number of environmental factors such as cytokines, growth factors, exosomes, and microRNAs impacting the immune cell response. Moreover, tumor cells in hostile microenvironments may activate their own intrinsic resistance mechanisms, such as autophagy, to escape the effective immune response. Such adaptive mechanisms may also include the ability of tumor cells to modify their metabolism and release several metabolites to impair the function of immune cells. In this review, we summarize the different mechanisms involved in the TME that affect the anti-tumor immune function of NK cells.

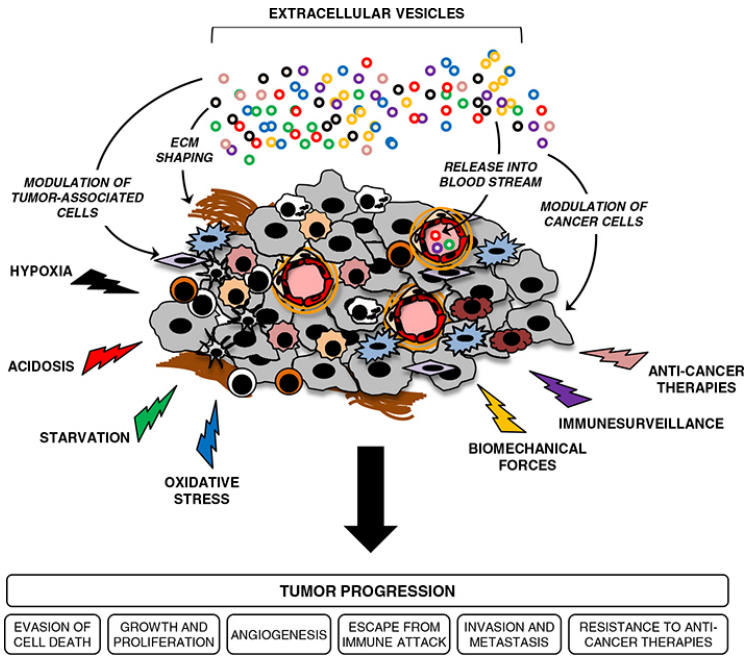
Baginska J, Viry E, Paggetti J, Medves S, Berchem G, Moussay E, Janji B. **The critical role of the tumor microenvironment in shaping natural killer cell-mediated anti-tumor immunity**. Front Immunol. 2013 Dec 25;4:490.



Heterotypic cellular interactions in the tumour microenvironment.

The **tumour microenvironment** is a complex scaffold of an **extracellular matrix** (ECM) and various cell types. In addition to malignant cells, **vascular cells, stromal cells and immune cells** are common cellular residents of the tumour niche. Tumour cells **mould this environment for their own needs** via intercellular communication pathways, such as **direct cell-to-cell contacts** and the **release of growth factors, matrix metalloproteases, ECM proteins and extracellular vesicles** (EVs). Tumour cell-mediated stromal modifications include: **suppression of anti-tumoural immune responses**, **deposition and degradation of ECM components**, induction of **vascular network** formation and **recruitment of stromal cells and tumour-promoting immune cells**. In turn, heterogeneous tumour microenvironmental components create a **favourable environment for tumour growth and dissemination**. Various tumour microenvironmental stressors are inherent features of solid tumours that profoundly modify the tumour milieu and accelerate tumour progression towards malignancy.

Kucharzewska P, Belting M. **Emerging roles of extracellular vesicles in the adaptive response of tumour cells to microenvironmental stress**. J Extracell Vesicles. 2013 Mar 5;2. doi: 10.3402/jev.v2i0.20304. eCollection 2013.



Extracellular vesicles (EVs) are potential conveyors of stress-mediated tumour progression.

EVs are shed from various cellular components of the tumour milieu to **mediate exchange of signalling proteins and genetic material**, which altogether may **support tumour growth and progression**. Diverse tumour microenvironmental **stress** conditions **augment tumour-promoting activities of EVs** by modulating their secretion and trafficking in the extracellular space, as well as altering their molecular content and functional activity. Upon release, **EVs may also enter the circulation and mediate long-range exchange of EV-associated cargo that may support the process of pre-metastatic niche formation**. In addition, circulating EVs carrying multifaceted, molecular stress signatures may offer unique, **non-invasive biomarkers** that can be used in the management of cancer patients.

Kucharzewska P, Belting M. **Emerging roles of extracellular vesicles in the adaptive response of tumour cells to microenvironmental stress**. J Extracell Vesicles. 2013 Mar 5;2. doi: 10.3402/jev.v2i0.20304. eCollection 2013.

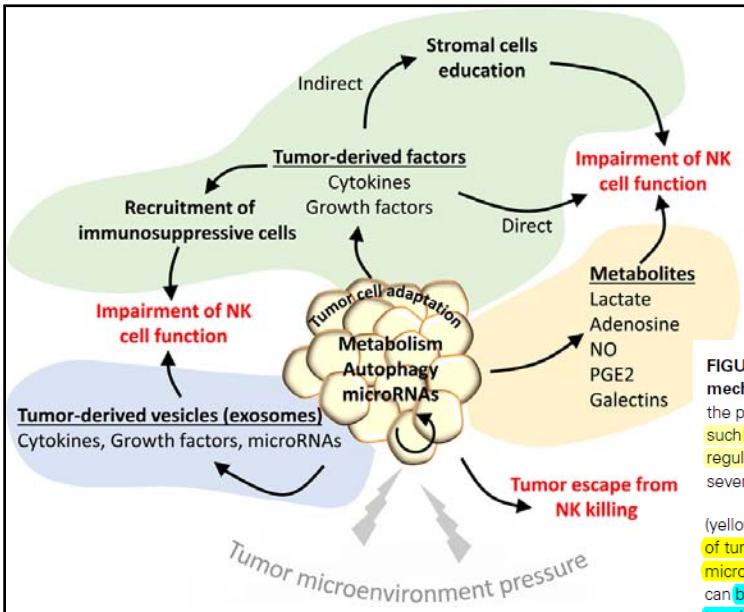
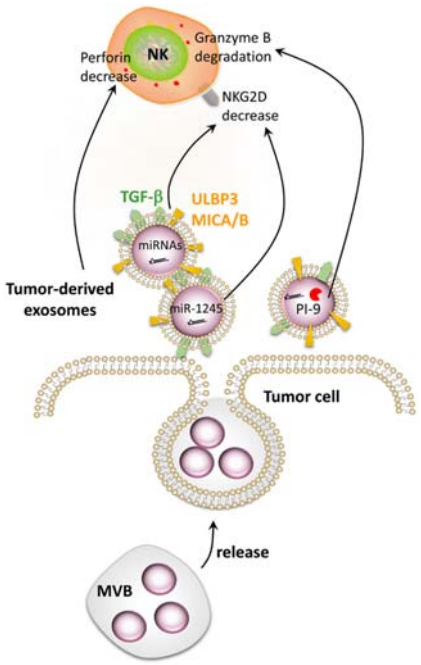


FIGURE 1 | The tumor microenvironment activates different mechanisms to impair the NK-mediated anti-tumor immunity. Under the pressure of the tumor microenvironment (TME), tumor cells adapt to such stress by activating intrinsic resistance mechanisms (autophagy) or by regulating their metabolism. Such regulation leads to the secretion of several metabolites that impair the function of NK cells in the tumor site (yellow area). Tumor cells under stress conditions may activate the release of tumor-derived vesicles containing cytokines, growth factors, or microRNAs to directly impact the NK functions (blue area). Such factors can be secreted directly in the TME to recruit immunosuppressive cells or to educate stromal cells involved in the impairment of NK cell functions (green area).

Baginska J, Viry E, Paggetti J, Medves S, Berchem G, Moussay E, Janji B. **The critical role of the tumor microenvironment in shaping natural killer cell-mediated anti-tumor immunity**. Front Immunol. 2013 Dec 25;4:490.



Impairment of NK cell function by tumor-derived exosomes. Tumor cells secrete extracellular vesicles called **exosomes**. Tumor-derived exosomes contain numerous **factors able to modulate the function of NK cells** such as MICA/B, ULBP3, TGF-β, PI-9, and different microRNAs. Exosome-derived MICA/B, ULBP3, TGF-β, and miR-1245 can decrease NKG2D on the surface of NK cells, while PI-9 degrades granzyme B. Tumor-derived exosomes can also **decrease the level of perforin in NK cells** by a still-unknown mechanism.

Baginska J, Viry E, Paggetti J, Medves S, Berchem G, Moussay E, Janji B. **The critical role of the tumor microenvironment in shaping natural killer cell-mediated anti-tumor immunity**. Front Immunol. 2013 Dec 25;4:490.

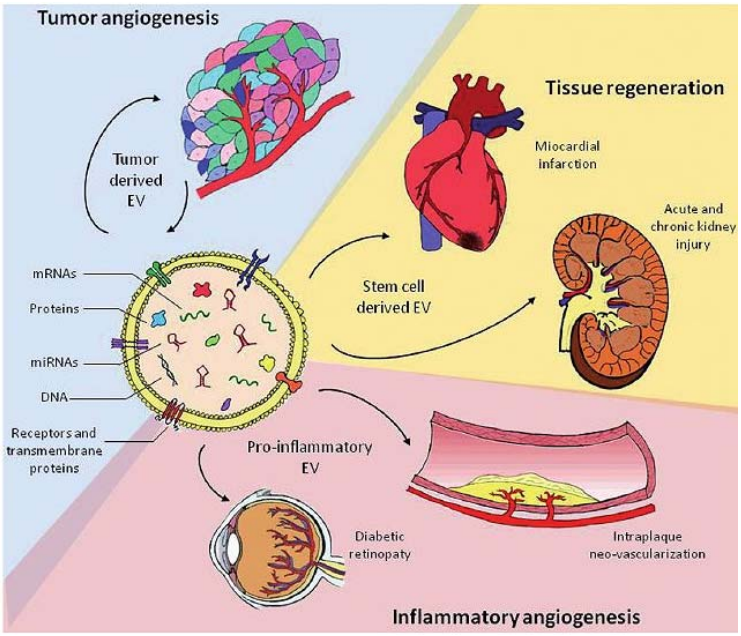
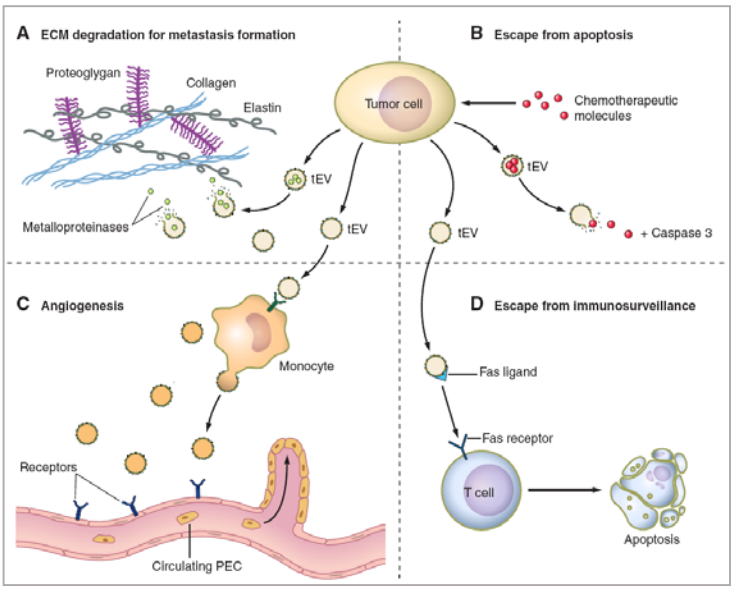


Fig. 1. Angiogenic potential of EV. EV by delivering their cargo to endothelial cells may be involved in angiogenic homeostasis, in the angiogenesis associated with tissue repair and in the altered angiogenesis occurring in tumors and inflammation.

Gai C, Carpanetto A, Deregibus MC, Camussi G. **Extracellular vesicle-mediated modulation of angiogenesis**. Histol Histopathol. 2016 Apr;31(4):379-91.



Turturici G, Tinnirello R, Sconzo G, Geraci F**. Extracellular membrane vesicles as a mechanism of cell-to-cell communication: advantages and disadvantages**. Am J Physiol Cell Physiol. 2014 Apr 1;306(7):C621-33.

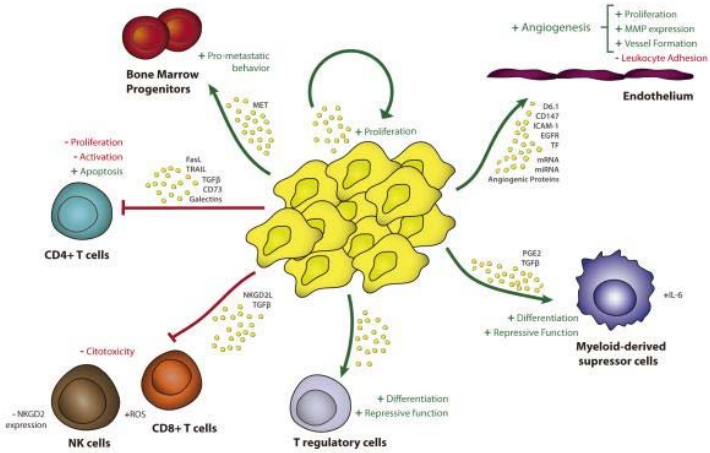
Le vescicole extracellulari (Evs) derivate dai tumori (tEVs) influenzano diversi aspetti della progressione tumorale.

A: Le EVs versate dai tumori contengono **metalloproteinasi** che sono responsabili dalla **degradazione della matrice** facilitando l'invasione tumorale;

B: le tEVs permettono alle cellule tumorali di **sopravvivere alla chemioterapia e all'apoptosi** mediante **efflusso dei farmaci e della caspasi 3,** rispettivamente;

C: Le tEVs stimolano la **secrezione di fattori pro-angiogenici** dalle cellule stromali e facilitano la proliferazione delle cellule endoteliali con ciò promuovendo l'angiogenesi e permettendo la crescita tumorale. L'angiogenesi è inoltre influenzata dal rilascio di **mRNA e miRNA** mediante tEVs.

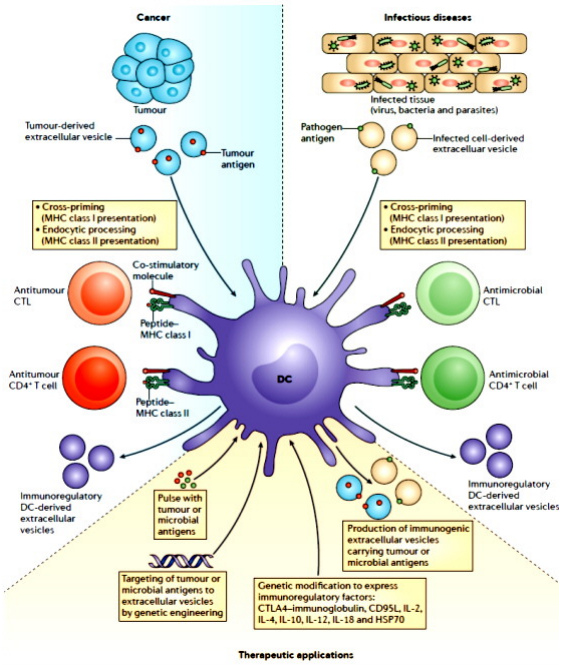
D: Le tEVs rilasciate da molte cellule tumorali espongono **Fas ligand**, che **induce l'apoptosi delle cellule T e inibisce la funzione delle cellule della risposta immunitaria adattativa** perciò permettendo alle cellule tumorali di evadere l'immunosorveglianza.



Gutiérrez-Vázquez C, Villarroya-Beltri C, Mittelbrunn M, Sánchez-Madrid F. **Transfer of extracellular vesicles during immune cell-cell interactions**. Immunol Rev. 2013 Jan;251(1):125-42.

Tumor-derived EVs promote tumor growth via multiple routes

Tumor-derived EVs suppress anti-tumor immune responses by inhibiting T-cell activation and proliferation and stimulating their apoptosis. EVs produced by tumor cells also induce regulatory T cells and MDSCs and inhibit the cytotoxicity of NK and CD8+ T cells Tumor-derived EVs are taken up by endothelial cells promoting angiogenesis and tumor invasion; the expression of CD147, D6.1A, tissue factor and EGFR in EVs, the transfer of pro-angiogenic components and the induction of MMPs play a role in the pro-angiogenic effects of tumor EVs Tumor EVs also contribute to tumor growth by stimulating tumor proliferation and inducing metastatic behavior in bone-marrow progenitors



RUOLO DELLE VESCICOLE EXTRACELLULARI NELLA REGOLAZIONE DELL'IMMUNITA' DEI TUMORI E DEI MICROORGANISMI CHE PUO' ESSERE MODIFICATA PER APPLICAZIONI TERAPEUTICHE

Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat Rev Immunol. 2014 Mar;14(3):195-208.

Contenuto e funzioni di vescicole extracellulari rilasciate da differenti tipi di cellule tumorali – 1

Tumor Type	Type of Vesicles	Content	Function
Lung carcinoma	Microvesicles	EMMPRIN	Tumor stroma interaction
Lung carcinoma	Microvesicles	None	Angiogenesis and metastasis
Lung carcinoma	Microvesicles	Lung specific RNAs	Phenotypic changes in marrow cells
Pancreatic adenocarcinoma, colorectal adenocarcinoma, lung carcinoma.	Microvesicles	mRNA for VEGF, HGF, IL-8 and surface determinants (CD44H)	Activation of tumor infiltrating monocytes
Prostate carcinoma	Microvesicles	Matrix metalloproteinases; Exchange of receptors (CX3CL1/fractalkine-CX3CR1)	Establishment of a favorable tumor niche
Prostate carcinoma	Microvesicles	Prostate specific RNAs	Prostate specific gene expression in human bone marrow cells.
Breast cancer	Exosomes	Hsp90alpha	Increase in cancer cell motility
Gliomas	Microvesicles	Oncogenic form of EGFRvIII	Tumor progression
Breast carcinoma and glioma cells	Microvesicles	Trans glutaminase, fibronectin	Transformation
Ovarian cancer	Microvesicles	CD147/extracellular matrix metalloproteinase inducer	Angiogenesis

Camussi G, Deregibus MC, Tetta C. **Tumor-derived microvesicles and the cancer microenvironment**. Curr Mol Med. 2013 Jan;13(1):58-67.

Contenuto e funzioni di vescicole extracellulari rilasciate da differenti tipi di cellule tumorali – 2

Tumor Type	Type of Vesicles	Content	Function
Human squamous carcinoma, alveolar basal epithelial adenocarcinoma and colon cancer	Microvesicles	Oncogenic EGFR	Angiogenesis by induction of autocrine VEGF production
Rat pancreatic adenocarcinoma	Exosomes	CD44v6	Lung metastasis
Human fibrosarcoma and prostate carcinoma	Microvesicles	Sphingomyelin	Angiogenesis
Glioblastoma	Microvesicles	mRNA, microRNA, proteins	Tumor growth and diagnostic bio markers
Colorectal carcinoma	Microvesicles	Cell cycle related mRNA	Angiogenesis
Glioblastoma, medulloblastoma, atypical teratoid rabdoid tumor and melanoma	Microvesicles	Retro-transposon elements, amplified oncogene sequences-	Tumor growth and progression
Renal cancer stem cells	Microvesicles	mRNA and microRNA	Angiogenesis, tumor invasion and metastasis
Glioblastoma	Exosomes	Mitochondrial DNA	Tumor progression

Camussi G, Deregibus MC, Tetta C. **Tumor-derived microvesicles and the cancer microenvironment**. Curr Mol Med. 2013 Jan;13(1):58-67.

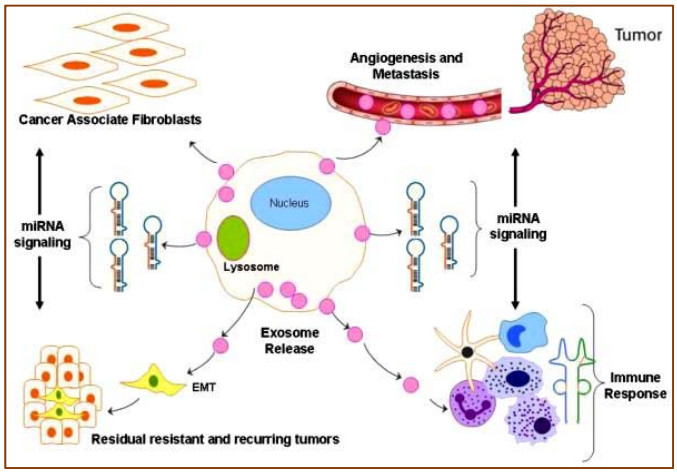
Contenuto e funzioni di vescicole extracellulari rilasciate da differenti tipi di cellule tumorali – 3

Tumor Type	Type of Vesicles	Content	Function	
Colorectal carcinoma	Microvesicles	Cell cycle related mRNA	Angiogenesis	[72]
Glioblastoma, medulloblastoma, atypical teratoid rabdoid tumor and melanoma	Microvesicles	Retro-transposon elements, amplified oncogene sequences-	Tumor growth and progression	[75]
Renal cancer stem cells	Microvesicles	mRNA and microRNA	Angiogenesis, tumor invasion and metastasis	[16]
Glioblastoma	Exosomes	Mitochondrial DNA	Tumor progression	[76]
Breast cancer	Exosomes	None	Conversion of MSCs in tumor associated myofibroblasts	[77]

Abbreviations: EMMPRIN, extracellular matrix metalloproteinase inducer also known as basigin and CD147; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; IL-8, interleukin-8; EGFR, epidermal growth factor receptor; MSCs, mesenchymal stem cells.

Camussi G, Deregibus MC, Tetta C. **Tumor-derived microvesicles and the cancer microenvironment**. Curr Mol Med. 2013 Jan;13(1):58-67.

Ruolo degli exosomi nel sostenere le reti di resistenza tumorale

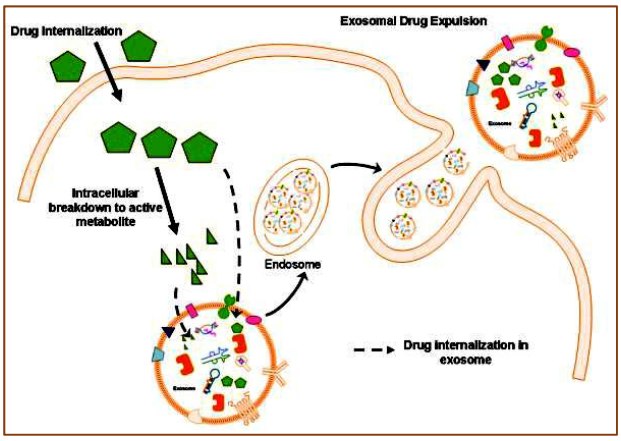


Azmi AS, Bao B, Sarkar FH. **Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review**. Cancer Metastasis Rev. 2013 Dec;32(3-4):623-42.

L'esportazione, mediata dagli exosomi, di materiale biologico può indurre un microambiente favorevole alla resistenza.

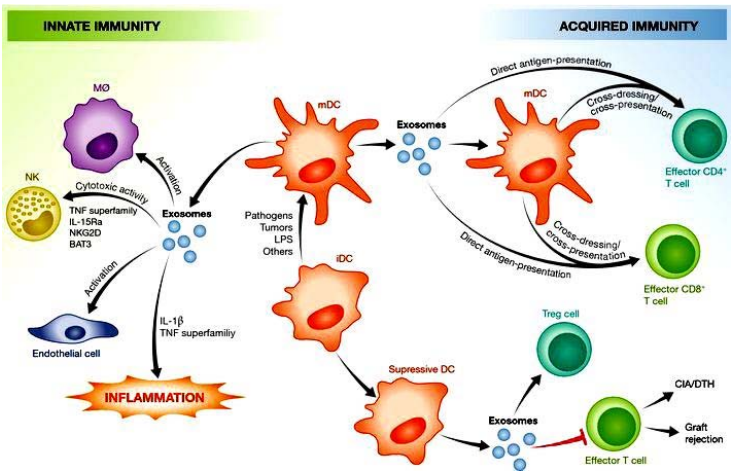
I fattori rilasciati dagli exosomi possono promuovere: (a) **morfologia cellulare tipo Epithelial-to-Mesenchymal Transition** (EMT), che dà origine a staminalità; b) promuovere la **formazione di cellule tipo fibroblastico** che provocano la reazione desmoplastica (reazione stromale); (c) promuovere **meccanismi di fuga immunitaria**; e (d) promuovere **angiogenesi** e **metastasi**. I **miRNAs** espulsi dagli exosomi possono regolare molteplici vie di segnalamento che promuovono cumulativamente un **fenotipo resistente** nella maggior parte dei tumori.

Meccanismi di estrusione dei farmaci - Ipotesi



Il diagramma illustra un "impacchettamento" intracellulare di farmaci chimici e/o dei loro prodotti di degradazione (forme attive). Tali **farmaci residenti negli exosomi possono essere espulsi dalle cellule** provocando una minore efficacia del farmaco; questo è un processo diverso dagli altri meccanismi di trasporto dei farmaci.

Azmi AS, Bao B, Sarkar FH. **Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review**. Cancer Metastasis Rev. 2013 Dec;32(3-4):623-42.

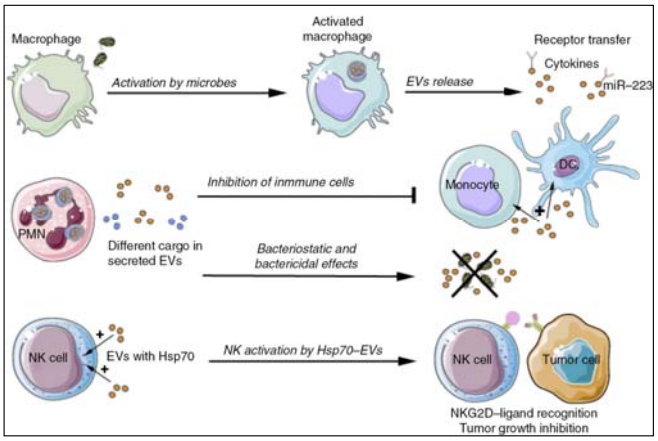


Microvescicole

Risposta immunitaria

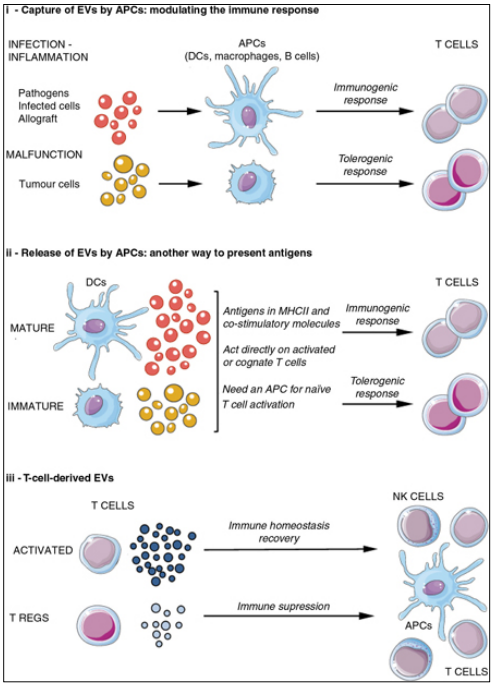
http://www.gene-quantification.de/exosomes.html

Ruolo fisiologico delle EVs derivate da cellule del Sistema immunitario innato



Activated macrophages release EVs that contain cytokines, miR-223 and carry out lateral transfer of receptors influencing myeloid cell proliferation and differentiation. Neutrophilic granulocytes (PMN) produce different types of EVs, depending on the type of stimulus. Neutrophil-derived EVs counteract the activation of immune cells or inhibit bacterial growth directly. EVs containing HSP-70 activate NK cells to combat tumour cells. DC = dendritic cell; NK = natural killer; NKG2D = natural killer group 2D; HSP = heat shock protein.

Yáñez-Mó M. **Biological properties of extracellular vesicles and their physiological functions**. J Extracell Vesicles. 2015 May 14;4:27066.



Le EVs nel Sistema Immunitario: presentazione di antigene e immunità acquisita

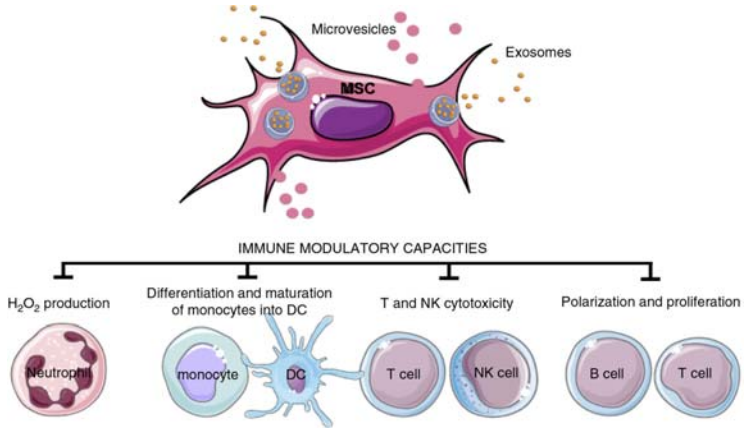
Le EVs possono giocare un ruolo sia nell'origine che nella progressione nella risposta immunitario acquisita, attuando a diversi livelli e su cellule diverse.

La figura reassume come le EVs sono coinvolte in tale processo.

APC: "antigen-presenting cell".
Treg: cellule T regolatorie.
NK: "natural Killer cell"
MHC: "major histocompatibility complex".

Yáñez-Mó M. **Biological properties of extracellular vesicles and their physiological functions**. J Extracell Vesicles. 2015 May 14;4:27066. doi: 10.3402/jev.v4.27066. eCollection 2015.

EVs derivate da cellule staminali mesenchimali (MSC)



Le EVs derivate dalle MSCs possono indurre effetti differenti a seconda della cellula bersaglio, come qui riassunto. **DC**: cellula dendritica; **NK**: "natural killer."

Yáñez-Mó M. **Biological properties of extracellular vesicles and their physiological functions**. J Extracell Vesicles. 2015 May 14;4:27066

Vescicole di membrana come vettori di risposte immunitarie

- **Porzioni della membrana plasmatica** di cellule coinvolte nella risposta immunitaria possono essere **trasferite fra cellule**, sia tramite contatto diretto (mediante i processi recentemente descritti di «**nibbling**» (rosicchiamento), **trogocitosi** e **nanotubi**) che tramite la ***secrezione di vescicole di membrana***.
- Le conseguenze funzionali di tali trasferimenti includono **l'induzione, amplificazione e/o modulazione delle risposte immunitarie nonché l'acquisizione di nuove proprietà funzionali da parte delle cellule che le ricevono, quali capacità migratorie o metastatiche**.
- Inoltre, nelle vescicole di membrana secrete sono stati identificati **mRNAs** e **microRNAs**, e ciò ha sollevato l'eccitante ipotesi che il **trasferimento di materiale genetico** potesse influenzare il comportamento delle cellule riceventi.
- Complessivamente, tali dati portano all'ipotesi che il **trasferimento di membrane sia un modo comune di comunicazione intercellulare**.

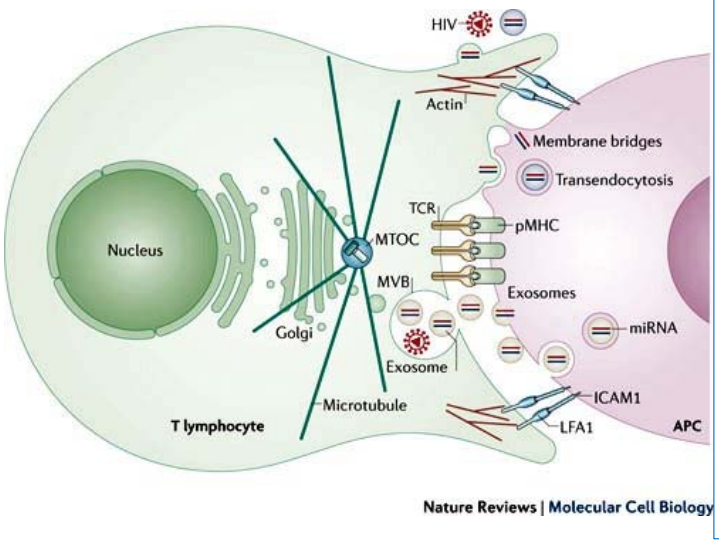
Théry C, Ostrowski M, Segura E. **Membrane vesicles as conveyors of immune responses**. Nat Rev Immunol. 2009 Aug;9(8):581-93.

Glossario (vedi figura Davis)

- **«Nibbling»** (rosicchiamento): Capacità che hanno le cellule dendritiche di strappare fisicamente frammenti di membrana da cellule vicine durante un contatto stretto senza indurre la morte della cellula donatrice.
- **Trogocitosi**: Trasferimento di frammenti della membrana plasmatica da una cellula ad un'altra senza indurre la morte cellulare. Questo processo è mediato da segnalamento mediato da recettore in seguito a contatto cellula-cellula.
- **Nanotubi**: Canale membranoso di 50-200 nm di diametro che collega cellule per lunghe distanze.
- **Vescicole di membrana**: Struttura sferica o approssimativamente sferiche limitata da un bilayer lipidico che racchiude un carico solubile.

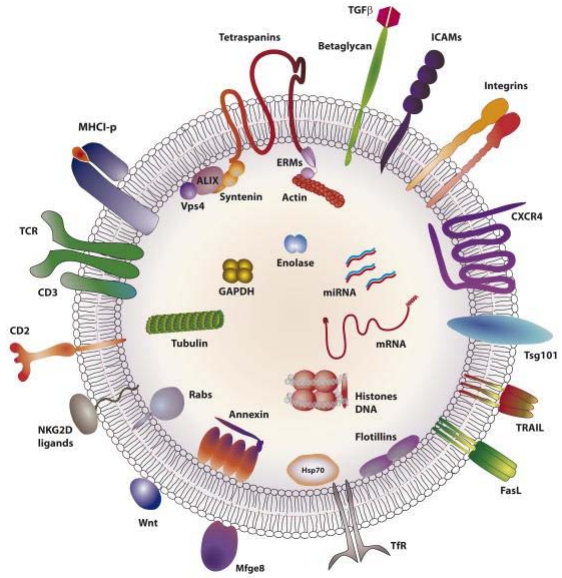
Théry C, Ostrowski M, Segura E. **Membrane vesicles as conveyors of immune responses**. Nat Rev Immunol. 2009 Aug;9(8):581-93.

La sinapsi immunologica funge da piattaforma per facilitare il passaggio di material genetico tra le cellule



- Durante la formazione di una **sinapsi immunologica**, le molecole coinvolte nel riconoscimento dell'antigene (ad es. il "T Cell Receptor; TCR) e le molecole del "peptide-loaded major histocompatibility complex; pMHC) si muovono verso un aggregato centrale circondato da un anello periferico arricchito in molecole di adesione (ad es. l'integrina "leukocyte function-associated antigen 1" (LFA1) e le "intercellular cell adhesion molecules; (ICAMs) e di citoscheletro di actina.
- Il linfocito T orienta il suo "microtubule-organizing centre (MTOC) e i compartimenti di secrezione (ad es. l'apparato di Golgi e "i multivesicular bodies» (MVBs) verso la "antigen presenting cell" (APC).
- Noi proponiamo **che la sinapsi immunologica fornisce una via di maggiore efficienza per lo scambio di materiale genetico** mediante la combinazione di differenti meccanismi, incluso la **secrezione polarizzata di exosomi** carichi di microRNA (miRNA), transendocitosi e ponti di membrana. I patogeni, incluso batteri e virus, si appropriano delle sinapsi biologiche per propagarsi da cellula a cellula.

Mittelbrunn M, Sánchez-Madrid F. **Intercellular communication: diverse structures for exchange of genetic information**. Nat Rev Mol Cell Biol. 2012 Apr 18;13(5):328-35.

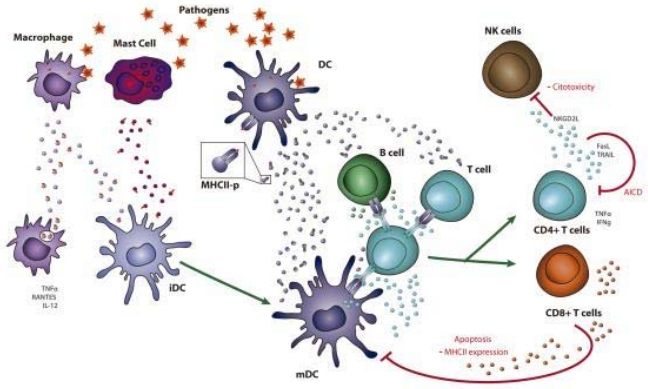


Typical molecular composition of T-cell exosomes

Membrane and luminal distribution of molecules predicted to be found in a typical exosome produced by a T lymphocyte. TCR: T-cell receptor; TGFβ: transforming growth factor beta; ICAMs: intercellular adhesion molecule family; CXCR4: C-X-C chemokine receptor type 4 or CD184; Tsg101: tumor susceptibility gene 101; TRAIL: TNF-related apoptosis-inducing ligand; FasL: Fas ligand or CD95L; TfR: transferrin receptor; Mfge8: milk-fat globule-EGF factor 8; ALIX: ALG-2-interacting protein X; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; ERMs: ezrin, radixin and moesin proteins. For more information about exosome composition see http://www.exocarta.org.

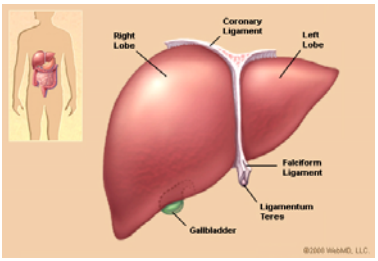
Gutiérrez-Vázquez C, Villarroya-Beltri C, Mittelbrunn M, Sánchez-Madrid F. **Transfer of extracellular vesicles during immune cell-cell interactions**. Immunol Rev. 2013 Jan;251(1):125-42.

Role of immune-cell-derived Evs during infection



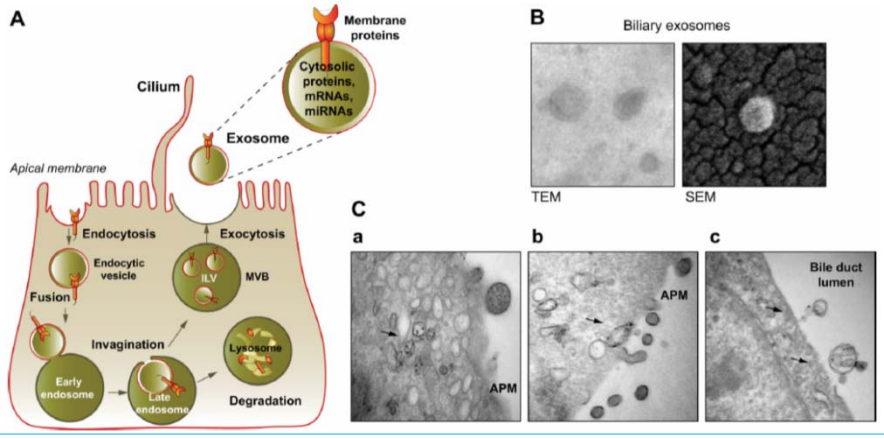
Gutiérrez-Vázquez C, Villarroya-Beltri C, Mittelbrunn M, Sánchez-Madrid F. **Transfer of extracellular vesicles during immune cell-cell interactions**. Immunol Rev. 2013 Jan;251(1):125-42.

During infection dendritic cells (DC) produce EVs that carry co-stimulatory molecules, antigens and Ag-MHC-II complexes. These EVs transfer Ag-presentation ability to other DCs and also to B cells and T cells , and might directly activate T cells. Mast cells and macrophages can also transfer Ag-containing EVs to DCs and induce maturation and presentation of the acquired Ags. EVs from macrophages also activate innate immune responses in uninfected macrophages. During Ag presentation, TCR and BCR triggering stimulate EV secretion, and the formation of a functional immune synapse promotes the functional transfer of EVs. Activated T cells produce immune-regulatory EVs that inhibit NK cytotoxicity, promote apoptosis in T cells and Ag-carrying DCs , and decrease DC antigen-presentation ability thus contributing to homeostasis recovery.



Vescicole extracellulari

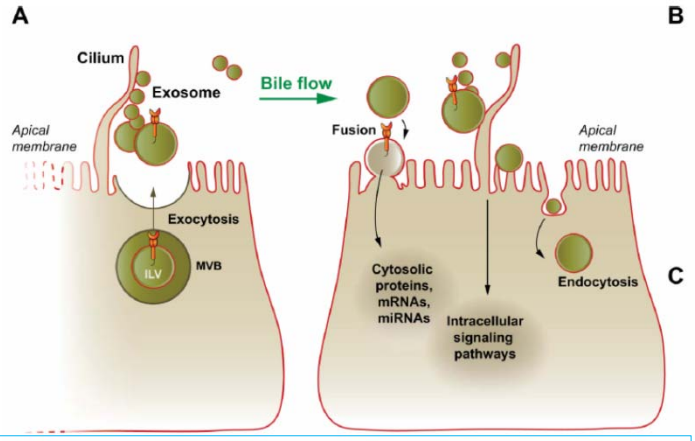
FEGATO



Exosome release

(A) **Exosomes** containing membrane and cytosolic proteins, mRNAs, and miRNAs, are **derived from the multivesicular body (MVB) sorting pathway**. **Membrane proteins are oriented in a fashion (extracellular region out) that permits profound biological autocrine and paracrine effects**. (B) Exosomes isolated from rat bile have a cup- or "deflated football"- shaped morphology by transmission electron microscopy (TEM), but they have a perfectly round shape by scanning electron microscopy (SEM). (C) In cholangiocytes of mouse liver, MVBs containing exosomes (arrows) (a) move to the apical plasma membrane (APM) (b), and release exosomes into the bile duct lumen by exocytosis (c).

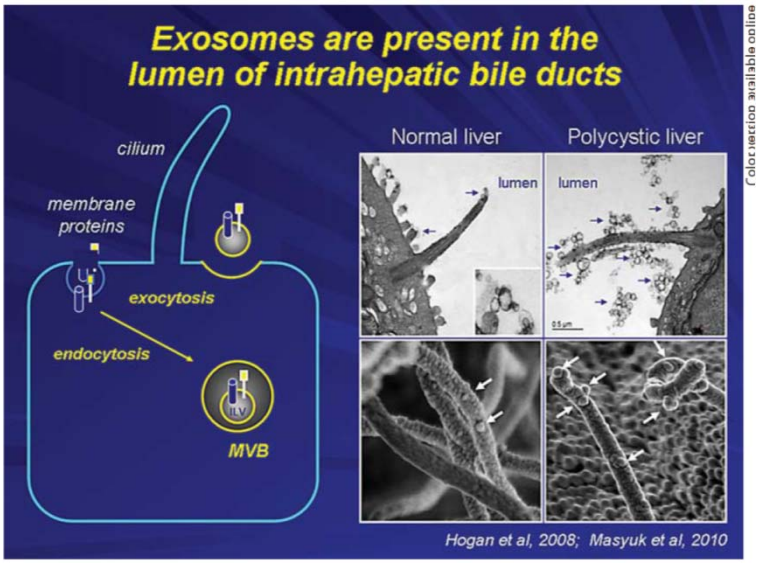
Masyuk AI, Masyuk TV, Larusso NF. Exosomes in the pathogenesis, diagnostics and therapeutics of liver diseases. J Hepatol. 2013 Sep;59(3):621-5.



Exosomes in intercellular signaling

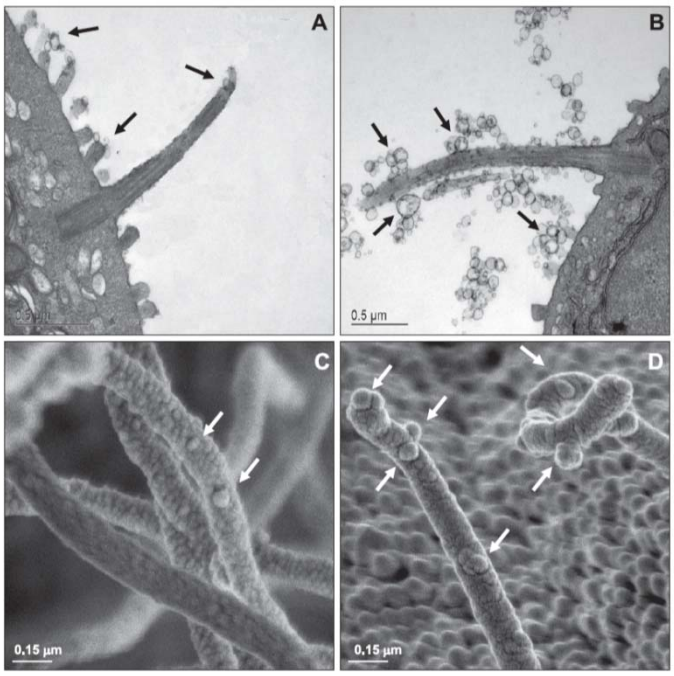
(A) In the liver, **exosomes derived from hepatocytes and cholangiocytes are transported by bile flow to target cholangiocytes with which they may interact via several mechanisms depending on their cargo and biological properties**. **They can fuse with the plasma membrane and deliver their content into the cytoplasm of a target cell**; **interact with receptors on the apical plasma and ciliary membrane inducing intracellular signaling**; and **endocytosed for recycling**. (B and C) Biliary exosomes surround and attach to cholangiocyte cilia in mouse liver as viewed by TEM (B) and SEM (C), supporting the involvement of exosomes and cilia in mechanisms of intercellular signaling.

Masyuk AI, Masyuk TV, Larusso NF. Exosomes in the pathogenesis, diagnostics and therapeutics of liver diseases. J Hepatol. 2013 Sep;59(3):621-5.



Larusso NF, Masyuk TV. **The role of cilia in the regulation of bile flow**. Dig Dis. 2011;29(1):6-12.

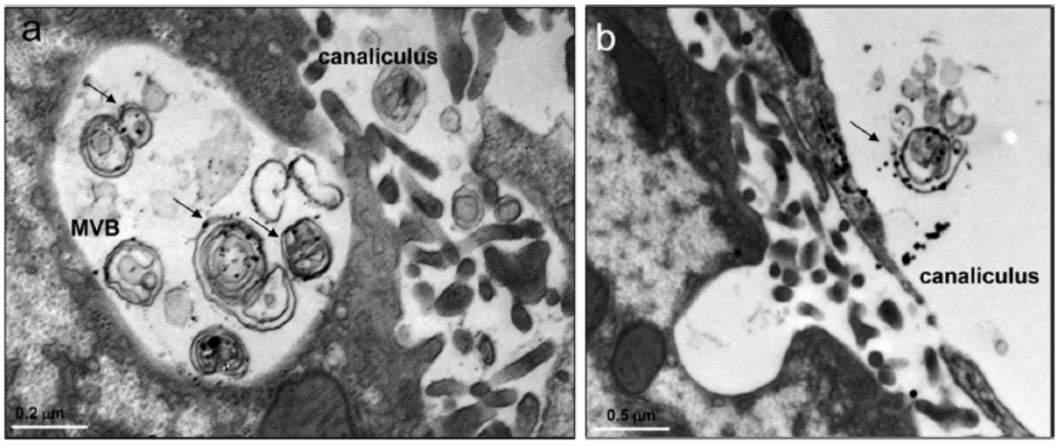
Gli **exosomi** sono **coinvolti nella funzione chemosensoriale delle cilia primarie dei colangiociti** (cellule dei dotti biliari). Gli exosomi sono piccole (30 -100 nm di diametro) vescicole extracellulari rivestite da membrana. Sono derivati da vescicole interne di corpi multivescicolari (MVBs) che si fondono con la membrana plasmatica in una modalità simile all'esocitosi e rilasciano il loro contenuto nello spazio extracellulare (schema). La presenza di vescicole tipo exosomi di 50-80 nm di diametro nel lume dei dotti intraepatici di topi «wild-type» e policistici è stata confermata da microscopia elettronica a trasmissione (destra, panelli di sopra). Queste vescicole circondono cilia dei colangiociti ed alcune sembrano attaccarsi alla membrana ciliare e dei microvilli. L'immagine del microscopio elettronico a scansione (SEM) (destra, panello di sotto) suggerisce che vescicole simili ad exosomi di fatto si leghino alle cilia.



Exosome-like vesicles surround and attach to mouse cholangiocyte [primary] cilia in vivo.

By transmission (TEM; A and B) and scanning (SEM; C and D) electron microscopy, exosome-like vesicles (black and white arrows) are present in the lumen of intrahepatic bile ducts in the wild-type (A and C) and Pkhd1del2/del2 (B and D) mice. The vesicles surround the cilium (B) and attach to this organelle (A–D) and microvilli (A) of the cholangiocyte apical plasma membrane.

Masyuk AI, Huang BQ, Ward CJ, Gradilone SA, Banales JM, Masyuk TV, Radtke B, Splinter PL, LaRusso NF. Biliary exosomes influence cholangiocyte regulatory mechanisms and proliferation through interaction with primary cilia. Am J Physiol Gastrointest Liver Physiol. 2010 Oct;299(4):G990-9.



Hepatocyte multivesicular bodies (MVBs) and **luminal vesicles are positive for an exosomal marker**, CD63 [tetraspanin].

MVBs and intraluminal vesicles positive for an exosomal marker, CD63, (black arrows) were observed in normal rat hepatocytes. MVBs are seen in a proximity to the hepatic canaliculus (a). CD63-positive vesicles are also seen in the canalicular lumen (b), suggesting that hepatocytes release exosomes in vivo.

Masyuk AI, Huang BQ, Ward CJ, Gradilone SA, Banales JM, Masyuk TV, Radtke B, Splinter PL, LaRusso NF. **Biliary exosomes influence cholangiocyte regulatory mechanisms and proliferation through interaction with primary cilia**. Am J Physiol Gastrointest Liver Physiol. 2010 Oct;299(4):G990-9.

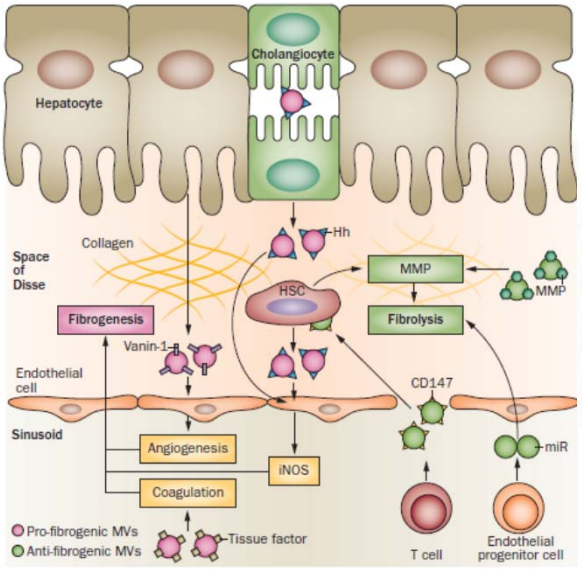


Figure 3 | Microvesicles in liver fibrosis. Some MVs (pink) promote fibrogenesis such as those produced by hepatocytes containing vanin-1 (which induce angiogenesis) or those that expose phosphatidylserine and/or tissue factor on their surface (activating coagulation). Cholangiocytes and HSCs release MVs containing Hh, which might also promote fibrogenesis by increasing iNOS expression. Other subpopulations of MVs decrease fibrosis (green). CD147-containing MVs released by T cells can be taken up into HSCs and upregulate MMP secretion. MVs containing MMP as well as miR-containing MVs released by endothelial progenitor cells might also promote fibrolysis. Abbreviations: Hh, Hedgehog ligand; HSC, hepatic stellate cell; iNOS, inducible nitric oxide synthase; miR, microRNA; MMP, matrix metalloproteinase; MV, microvesicle.

Lemoinne S, Thabut D, Housset C, Moreau R, Valla D, Boulanger CM, Rautou PE. **The emerging roles of microvesicles in liver diseases**. Nat Rev Gastroenterol Hepatol. 2014 Jun;11(6):350-61.

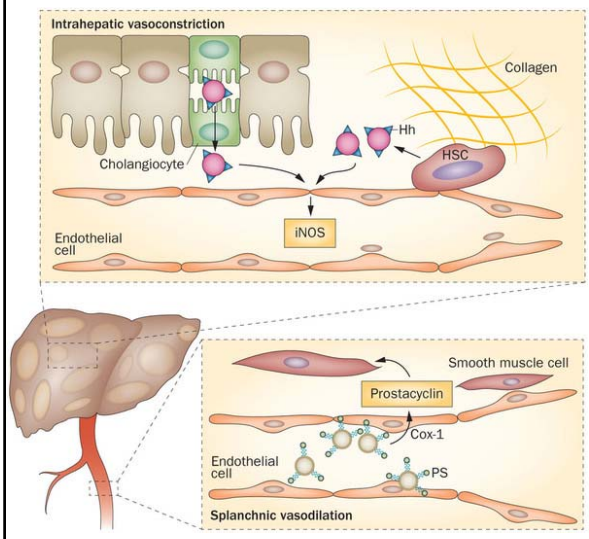


Figure 4 | Microvesicles in portal hypertension. Upper insert: In the cirrhotic liver, cholangiocytes and HSCs produce MVs containing Hh. These Hh-containing MVs induce endothelial expression of iNOS, probably contributing paradoxically to the intrahepatic vasoconstriction associated with cirrhosis. Vascular resistance is also increased by collagen deposition and contraction of HSCs, wrapped around endothelial cells. Lower insert: In cirrhosis, the splanchnic vascular bed is dilated. Levels of leukoendothelial, lymphocyte, erythrocyte and hepatocyte MVs are increased in the systemic circulation. These MVs expose PS at their surface that can be transferred (with other membrane phospholipids) to endothelial cells. Phospholipids are then used as substrates for the arachidonic acid pathway, including Cox-1 (which is an enzyme involved in the formation of vasodilator agents, such as prostacyclin), leading to smooth muscle cell relaxation. Abbreviations: Cox-1, cyclo-oxygenase-1; Hh, Hedgehog ligand; HSC, hepatic stellate cell; iNOS, inducible nitric oxide synthase; MV, microvesicle; PS, phosphatidylserine.

Lemoinne S, Thabut D, Housset C, Moreau R, Valla D, Boulanger CM, Rautou PE. **The emerging roles of microvesicles in liver diseases**. Nat Rev Gastroenterol Hepatol. 2014 Jun;11(6):350-61.

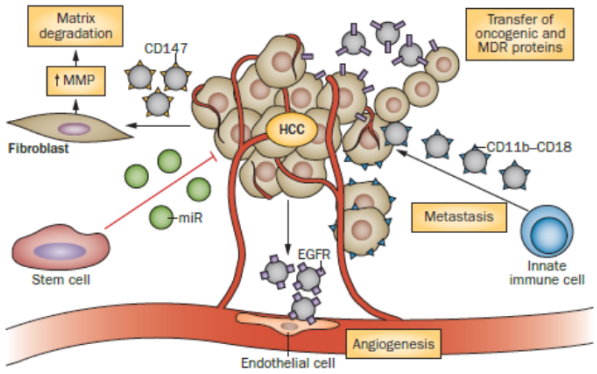


Figure 5 | Microvesicles in hepatocellular carcinoma. HCC cells might release MVs that interact with neighbouring cells to promote carcinogenesis. Tumoral stroma contains fibroblasts, which can favour tumoral progression. Tumour MVs containing CD147 induce upregulation of MMPs in fibroblasts, leading to extracellular matrix degradation and tumoral invasion. Tumour MVs can also transfer oncogenic forms of growth factor receptor or multidrug-resistant proteins to other tumour cells, thus propagating an increased proliferative, survival, and/or mitogenic capacity. In addition, EGFR, which is upregulated in HCC, could be transferred via tumour MVs to endothelial cells to promote angiogenesis to vascularize the growing tumour. Non-tumour cells can promote HCC metastasis via release of MVs: innate immune cells produce MVs harbouring CD11b–CD18, which can be taken up by HCC cells and promote their capacity of migration, invasion, attachment to the endothelium and metastasis. Non-tumour cells can also produce MVs with antitumoral properties. For instance, stem cells release MVs containing miR that inhibit proliferation of hepatic tumour cells. Abbreviations: HCC, hepatocellular carcinoma; MDR, multidrug resistant; MMP, matrix metalloproteinase; miR, microRNA; MV, microvesicle.

Lemoinne S, Thabut D, Housset C, Moreau R, Valla D, Boulanger CM, Rautou PE. **The emerging roles of microvesicles in liver diseases**. Nat Rev Gastroenterol Hepatol. 2014 Jun;11(6):350-61.

A niche role for cancer exosomes in metastasis

Yun Zhang and Xiao-Fan Wang

NATURE CELL BIOLOGY VOLUME 17 | NUMBER 6 | JUNE 2015

Cancer cells are known to secrete exosomes with pro-metastatic effects. Pancreatic-cancer-derived exosomes are now shown to promote liver metastasis by eliciting pre-metastatic niche formation through a multi-step process. This involves uptake of exosome-derived factors by liver Kupffer cells and hepatic stellate cell activation to generate a fibrotic microenvironment with immune cell infiltrates that favours metastasis.

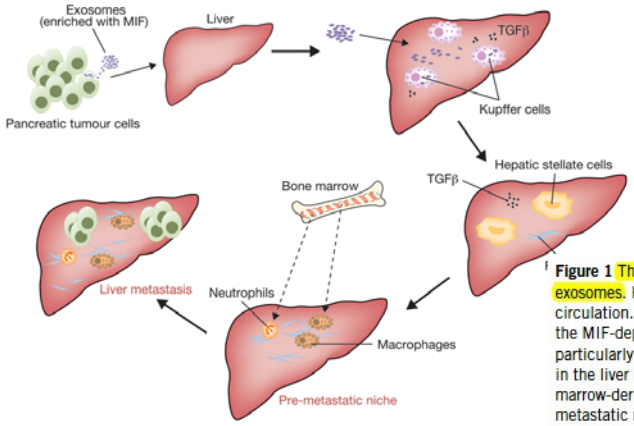
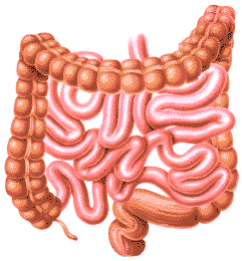
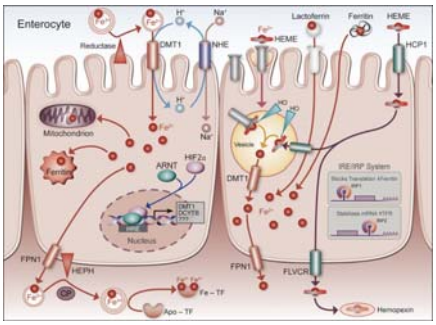


Figure 1 The sequential steps in liver pre-metastatic niche formation induced by PDAC-derived exosomes. Primary pancreatic tumour cells release exosomes that contain MIF into the blood circulation. These PDAC-derived exosomes are selectively taken up by liver Kupffer cells leading to the MIF-dependent production of fibrotic cytokines by the Kupffer cells. These fibrotic cytokines, particularly TGFβ, activate liver hepatic stellate cells to produce fibronectin. Deposition of fibronectin in the liver leads to the formation a fibrotic microenvironment that promotes the recruitment of bone marrow-derived cells such as macrophages and neutrophils. These sequential events establish a pre-metastatic niche, which permits the survival and proliferation of disseminated PDAC cells and the formation of metastases in the liver.

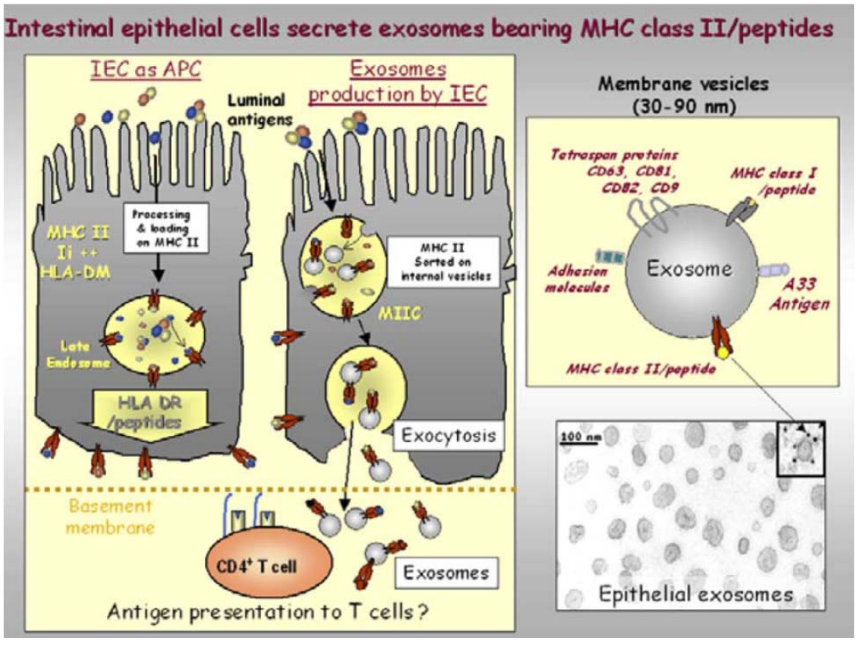




Vescicole extracellulari

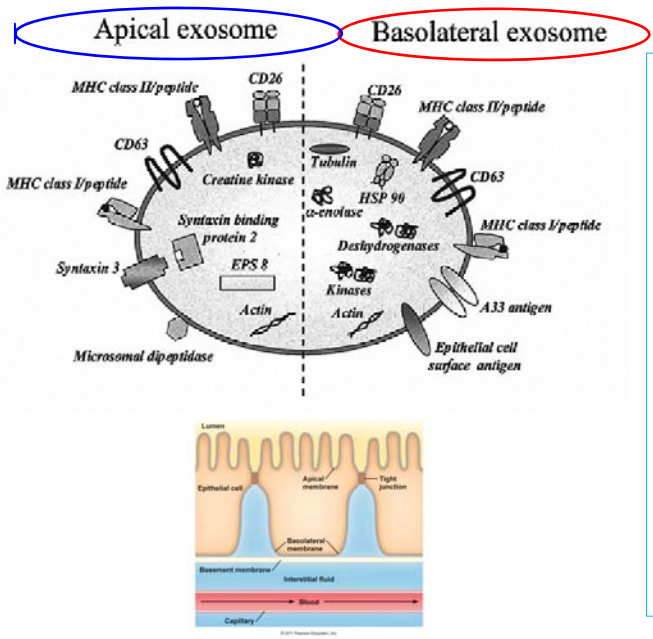
Intestino

http://aasarts.com/wp-content/uploads/2014/06/Enterocyte-Final.jpg



Intestinal epithelial cells (IEC) secrete exosomes.
IEC express accessory molecules (MHC class II, invariant chain, HLA-DM) and are considered as non-professional antigen presenting cells. The lack of direct contact between IEC and CD4+ T cells limits direct antigen presentation in vivo. However, IEC secrete exosomes which are small membrane vesicles originating from the MHC class II-enriched compartment (MIIC) and are released by exocytosis of these compartments in the external medium. Such epithelial exosomes bear class II/peptide complexes and molecules potentially involved in cell– cell or cell –matrix interactions.

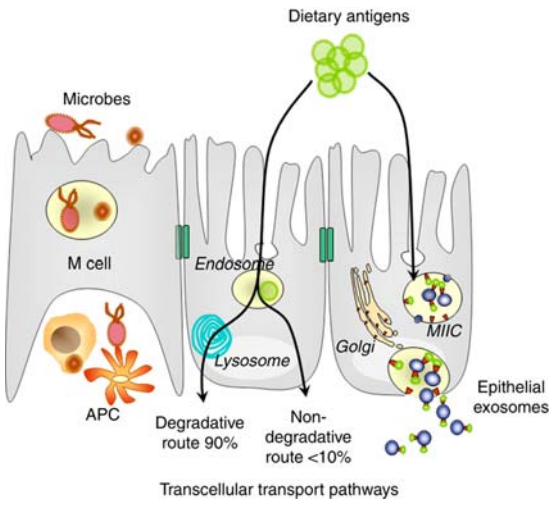
Mallegol J, van Niel G, Heyman M. **Phenotypic and functional characterization of intestinal epithelial exosomes**. Blood Cells Mol Dis. 2005 Jul-Aug;35(1):11-6.



A model for the molecular structure of epithelial-derived exosomes. Ubiquitously expressed molecules such as enzymes of the intracellular metabolism (pyruvate kinase M2, creatine kinase, α-enolase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, L-lactate dehydrogenase) and cytoskeleton proteins (actin, tubulin), as well as molecules possibly involved in antigen presentation (MHC class I, MHC class II, CD63), were found in both **apical** and **basolateral exosomes**. Apical exosomes also carried molecules involved in apical addressing of endosomes (syntaxin 3, syntaxin-binding protein 2), whereas basolateral exosomes had molecules that might act as adhesion or costimulatory molecules (A33 antigen and epithelial cell surface antigen).

van Niel G, Raposo G, Candalh C, Boussac M, Hershberg R, Cerf-Bensussan N, Heyman M. **Intestinal epithelial cells secrete exosome-like vesicles**. Gastroenterology. 2001 Aug;121(2):337-49.

INTESTINO
VIE DI TRASPORTO PARACELLULARE



Under steady-state condition, molecules of molecular weight (MW) > 600 Da (such as food antigens, peptides) are sampled by the epithelial cells by **endocytosis** at the apical membrane and **transcytosis** toward the lamina propria.

During transcytosis, full-length peptides or proteins are partly degraded in acidic and lysosomal compartments and released in the form of amino acids (total degradation) or breakdown products (partial degradation) at the basolateral pole of enterocytes. Early endosomes containing partially degraded food antigens meet the major histocompatibility complex (MHC) class II-enriched compartment (MIIC) where exogenous peptides are loaded on MHC class II molecules. Inward invagination of MIIC compartment lead to the formation of **exosomes**, which are small membrane vesicles (40 – 90 nm) bearing MHC class II / peptide complexes at their surface. **Exosomes can diffuse in the basement membrane and interact with local immune cells**. **Exosome-bound peptides are much more potent than free peptides to interact with dendritic cells and stimulate peptide presentation to T cells.**

Ménard S, Cerf-Bensussan N, Heyman M. **Multiple facets of intestinal permeability and epithelial handling of dietary antigens**. Mucosal Immunol. 2010 May;3(3):247-59.

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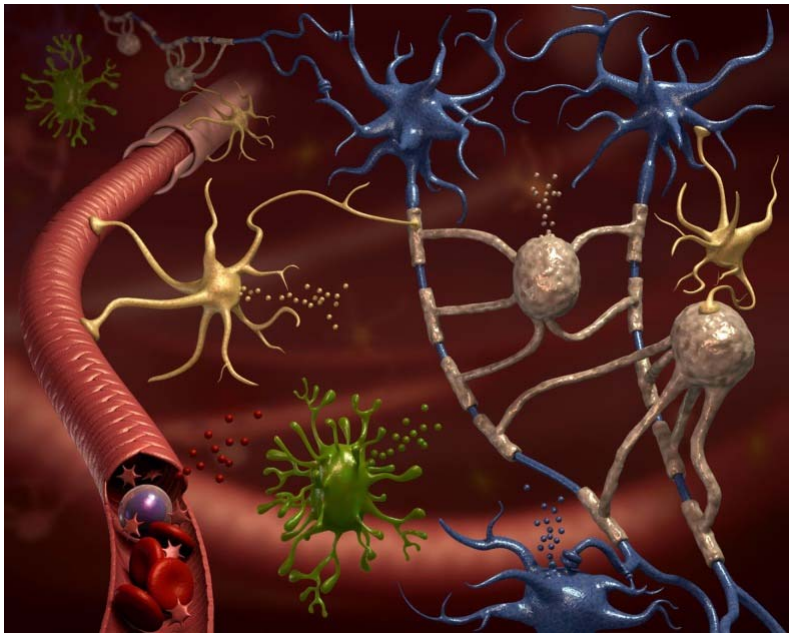
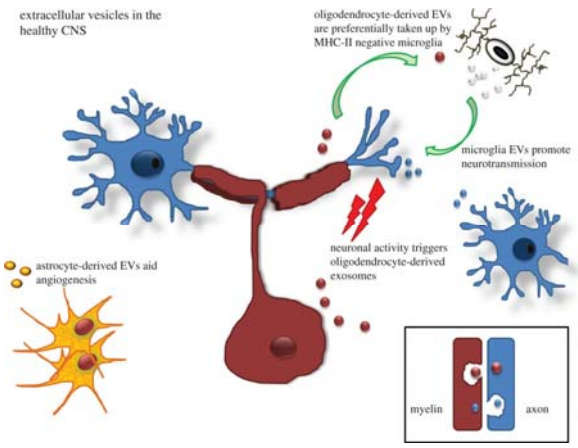
Neuroimmunologia clinica
Unità INSPE
Ospedale San Raffaele, Milano

Vescicole extracellulari

Cervello



Colombo E, Borgiani B, Verderio C, Furlan R. **Microvesicles: novel biomarkers for neurological disorders**. Front Physiol. 2012 Mar 29;3:63.
http://renderingedisegno.blogspot.it/2012/04/blog-post.html



The role of extracellular vesicles in the healthy CNS.

EVs carry signatures of the cell in question as well as specific EV-related factors. The impact of such release depends upon the cell type releasing the EVs and the cell type taking up the particles

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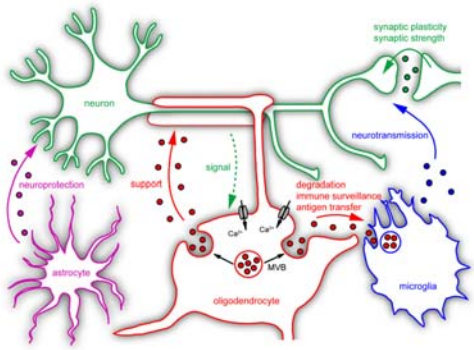
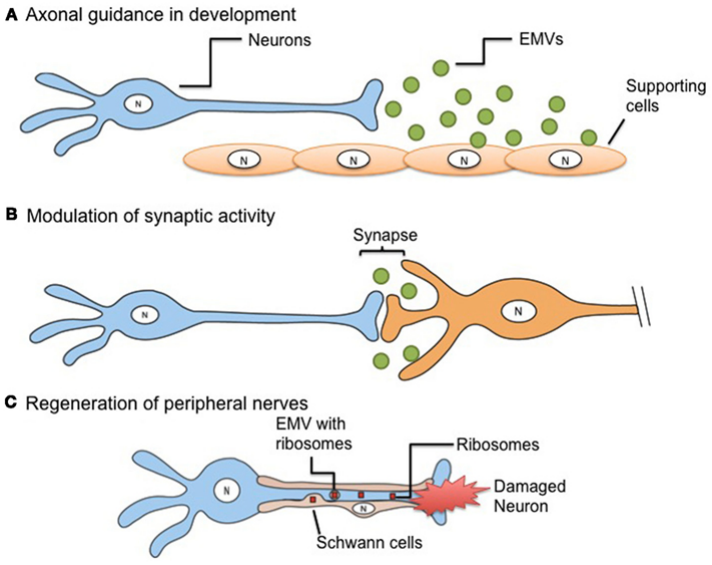


Postulated roles of microvesicles in neural cell communication. Neural cells release different types of microvesicles with several known or suggested functions. **Neurons** secrete exosomes which may influence synaptic plasticity. **Microglia** modulate neurotransmission via shedding microvesicles. **Astrocyte**-derived exosomes carry neuroprotective cargo and could contribute to neuronal survival. Neuronal signals trigger exosome release from oligodendrocytes by raising intracellular Ca^{2+}-levels. Upon internalization by neurons these exosomes could provide support to axons. Microglia take up and degrade oligodendroglial exosomes without changing their inflammatory properties. Under specific pathological conditions these exosomes may transfer antigens to microglial cells or other APCs and induce inflammatory responses.

Frühbeis C, Fröhlich D, Krämer-Albers EM. **Emerging roles of exosomes in neuron-glia communication**. Front Physiol. 2012 Apr 30;3:119.



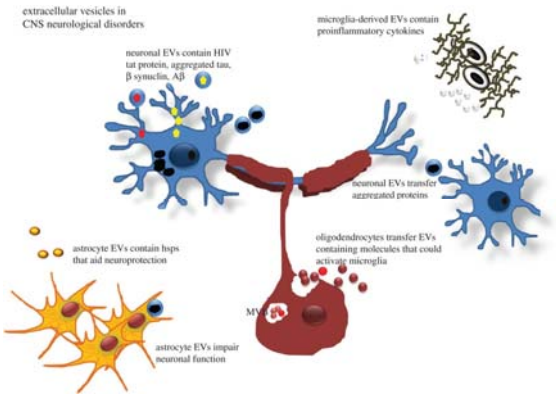
Extracellular membrane vesicles-mediated mechanisms in neurons.

(A) A gradient of EMVs in the developing nervous system can serve as a directional guide to axonal growth.

(B) EMVs released from presynaptic nerve terminals and taken up by their postsynaptic partners can carry informational content which can modulate the strength of synaptic activity.

(C) Regeneration of peripheral nerves is enhanced by the EMV transfer of ribosomes and mRNA directly from surrounding Schwann cells into the injured nerve to promote protein synthesis.

Lai CP, Breakefield XO. **Role of exosomes/microvesicles in the nervous system and use in emerging therapies**. Front Physiol. 2012 Jun 27;3:228.



Extracellular vesicles in neurological disorders: proposed actions. In neurodegenerative disorders, neurons, and in some cases astrocytes, produce and release aggregated proteins such as α-synuclein, APP and phosphorylated tau and, in the case of prion disorders, pathogenic PrPSc protein. The EVs released may act as 'seeds' that spread the damage throughout the brain. In demyelinating disease, myelin-stressed oligodendrocytes produce altered myelin proteins and heat shock proteins (hsps) that may (hypothetically) be released in EVs. The 'disease-associated' proteins activate microglia that may augment disease or alternatively affect neurons and axons leading to dysfunction.

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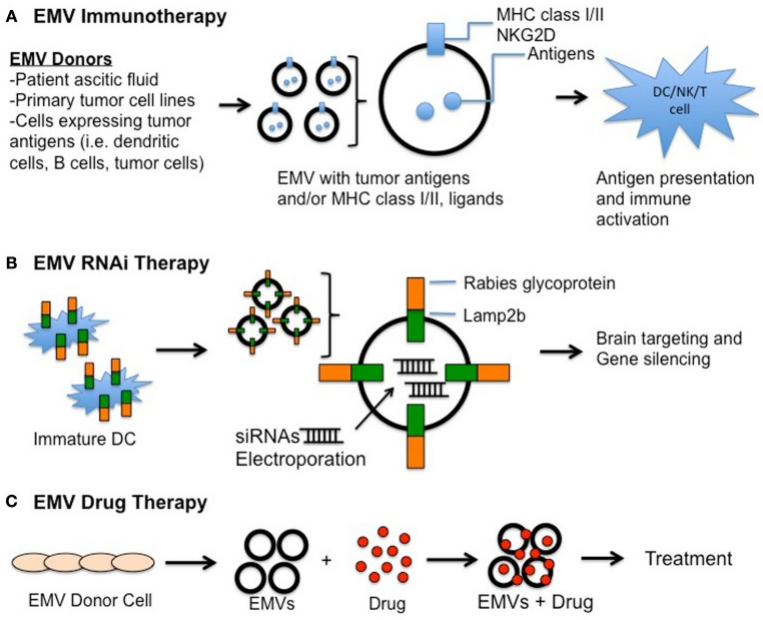
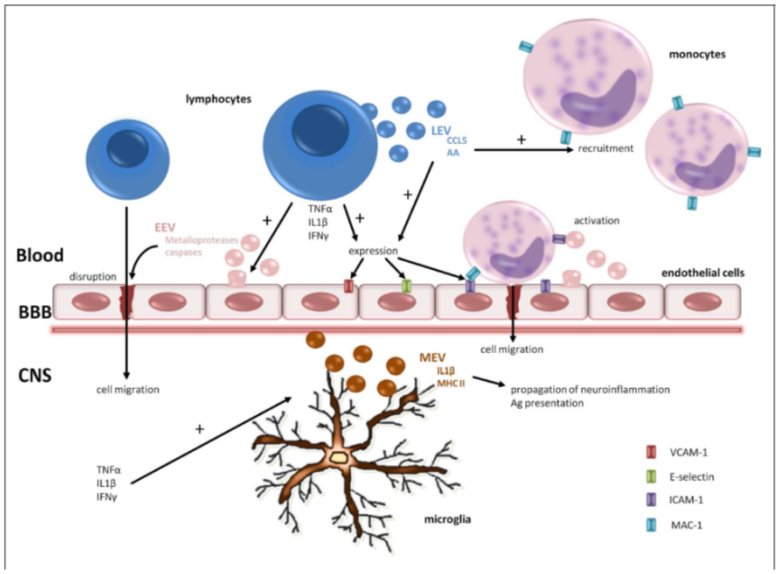


Lai CP, Breakefield XO. **Role of exosomes/microvesicles in the nervous system and use in emerging therapies**. Front Physiol. 2012 Jun 27;3:228.



Extracellular membrane vesicles-based therapies.

(A) EMV immunotherapy. EMVs containing tumor-antigen within and/or on the membrane surface are isolated from different sources and introduced in vivo to elicit targeted immuneresponses.

(B) EMV RNAi therapy. EMVs derived from immature dendritic cells (DCs) expressing Rabies glycoprotein-Lamp2b fusion protein were electroporated with siRNAs for targeting against neurons, microglia, and oligodendrocytes for subsequent gene silencing.

(C) EMV drug therapy. Therapeutic compounds can be packaged into/onto EMVs isolated from donor cells to minimize degradation and increase delivery to intended sites.

EMV: extracellular membrane vesicles

Sáenz-Cuesta M, Osorio-Querejeta I, Otaegui D. **Extracellular Vesicles in Multiple Sclerosis: What are They Telling Us?** Front Cell Neurosci. 2014 Mar 28;8:100.

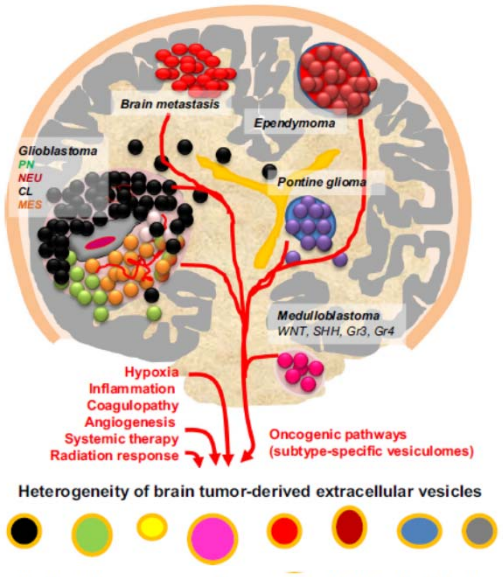


Fig. 1 Heterogeneity of extracellular vesicle profiles in brain tumors. Several processes may influence the heterogeneity of extracellular vesicles (EVs) produced by brain tumors. This includes heterogeneity of disease entities and of cancer cells, diversity of vesiculation processes driven by intrinsic cellular networks, including oncogenic pathways, and their modulation by the microenvironment and therapeutic interventions. As detailed in the text, EV heterogeneity encompasses emission rates, types of EVs released (exosomes, ectosomes, other vesicles), their molecular cargo (oncogenic proteins and nucleic acids, effector molecules), and biological activities. Tumor stoma may also contribute to the unique signature and function of EVs in brain tumors. For example glioblastoma (GBM) exists in four molecular subtypes (PN, NEU, MES, CL) driven by different oncogenic pathways, which includes different expression profiles of vesiculation-regulating genes (vesiculomes) (Nakano et al. 2015)

Hypothesis – the emergence of context-specific diversity in brain tumour vesiculation: *Different brain tumour types may produce EVs with different biogenetic origin, molecular composition, oncogenic cargo, biological activities and roles in tumor progression. Regulatory effects of oncogenic mutations, microenvironment and therapy likely modulate the EV repertoire (vesiculome) in brain tumours.*

D'Asti E, Chennakrishnaiah S, Lee TH, Rak J. **Extracellular Vesicles in Brain Tumor Progression**. Cell Mol Neurobiol. 2016 Mar 18. [Epub ahead of print]

CENTRE OF EXCELLENCE ON NEURODEGENERATIVE DISEASES

Objectives: We have described cerebrospinal fluid (CSF) myeloid microvesicles (MVs) as a marker of microglia activation during neuroinflammation in Alzheimer disease (AD), and characterized their ability to produce toxic amyloid β_{1-42} ($A\beta_{1-42}$) oligomers from aggregated or soluble substrate. The aim of this study is to investigate the association of CSF myeloid MVs with neuroimaging, clinical, and paraclinical data in AD and mild cognitive impairment (MCI).
Methods: We collected CSF from 106 AD patients, 51 MCI patients, and 29 neurologically healthy controls. We examined CSF myeloid MV content and AD markers. A subgroup of 34 AD and 21 MCI patients underwent structural and diffusion tensor MRI.
Results: Higher levels of myeloid MVs were found in the CSF of AD patients and MCI patients converting within 3 years relative to controls, but also, at a lower level, in MCI patients not converting to AD. CSF myeloid MVs were associated with Tau but not with $A\beta_{1-42}$ CSF levels. CSF MVs levels correlated with white matter (WM) tract damage in MCI, and with hippocampal atrophy in AD.
Interpretation: Microglial MVs are neurotoxic and myelinotoxic in the presence of $A\beta_{1-42}$. CSF myeloid MVs, mirroring microglia activation and MV release, are associated with WM damage in MCI and hippocampal atrophy in AD. This suggests that hippocampal microglia activation, in the presence of $A\beta_{1-42}$ in excess, produces neurotoxic and oligodendrotoxic oligomers that, through WM tract damage, spread disease to neighboring and connected areas, causing local microglia activation and propagation of disease through the same sequence of events.

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Agosta F, Dalla Libera D, Spinelli EG, Finardi A, Canu E, Bergami A, Bocchio Chiavetto L, Baronio M, Comi G, Martino G, Matteoli M, Magnani G, Verderio C, Furlan R. **Myeloid microvesicles in cerebrospinal fluid are associated with myelin damage and neuronal loss in mild cognitive impairment and Alzheimer disease.** Ann Neurol. 2014 ec;76(6):813-25.

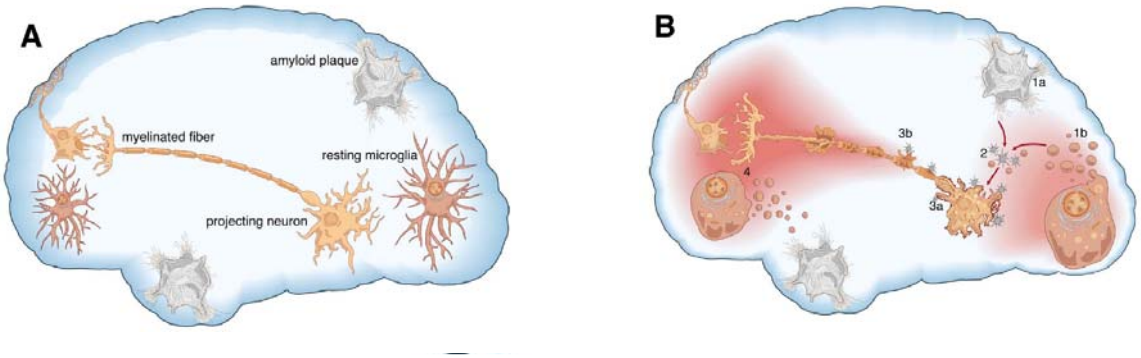

FIGURE 6: (A) For the mechanism of disease propagation that we hypothesize, the crucial players in the brain of Alzheimer disease (AD) patients are amyloid plaques, resting microglia, projecting neurons, and myelinated projecting fibers. (B) AD is associated with the deposition of extracellular amyloid β plaques (1a). Microglia activation is associated to the production of myeloid microvesicles (MMVs; 1b), which results in an increased production of oligomeric $A\beta_{1-42}$ (2), leading to both neurotoxicity (3a) and oligodendrotoxicity (3b). Activation of microglia can also induce the hyperphosphorylation and aggregation of Tau, also leading to neuronal damage (3a). The damage caused on both neurons and oligodendrocytes by MMVs results in degeneration of projecting fibers from the initially involved to more distal areas (3b), causing the diffusion of the pathogenic process to new brain regions (4).

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Microvesicles: what is the role in multiple sclerosis?

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Microvesicles are a recently described way of cell communication that has been implicated in a number of biological processes, including neuroinflammation. Widely investigated as biomarkers in oncology and neurological disorders, little is known of the role of microvesicles in the pathogenesis of diseases such as multiple sclerosis (MS). Several evidences suggest that pro-inflammatory microglia and infiltrating macrophages release microvesicles that spread inflammatory signals and alter neuronal functions. We review here available information on microvesicles, with a special focus on microglia and macrophage microvesicles, in the pathogenesis of MS, and as potential biomarkers and therapeutic targets.

Carandini T, Colombo F, Finardi A, Casella G, Garzetti L, Verderio C, Furlan R. **Microvesicles: What is the Role in Multiple Sclerosis?** Front Neurol. 2015 May 26;6:111.

Microvesicles: novel biomarkers for neurological disorders

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Microvesicles (MVs) are released by most cell types in physiological conditions, but their number is often increased upon cellular activation or neoplastic transformation. This suggests that their detection may be helpful in pathological conditions to have information on activated cell types and, possibly, on the nature of the activation. This could be of paramount importance in districts and tissues that are not accessible to direct examination, such as the central nervous system. Increased release of MVs has been described to be associated to the acute or active phase of several neurological disorders. While the subcellular origin of MVs (exosome or ectosomes) is basically never addressed in these studies because of technical limitations, the cell of origin is always identified. Endothelium- or platelet-derived MVs, detected in plasma or serum, are linked to neurological pathologies with a vascular or ischemic pathogenic component, and may represent a very useful marker to support therapeutic choices in stroke. In neuroinflammatory disorders, such as multiple sclerosis, MVs of oligodendroglial, or microglial origin have been described in the cerebrospinal fluid and may carry, in perspective, additional information on the biological alterations in their cell of origin. Little specific evidence is available in neurodegenerative disorders and, specifically, MVs of neural origin have never been investigated in these pathologies. Few data have been reported for neuroinfection and brain trauma. In brain tumors, despite the limited number of studies performed, results are very promising and potentially close to clinical translation. We here review all currently available data on the detection of MVs in neurological diseases, limiting our search to exclusively human studies. Current literature and our own data indicate that MVs detection may represent a very promising strategy to gain pathogenic information, identify therapeutic targets, and select specific biomarkers for neurological disorders.

Colombo E, Borgiani B, Verderio C, Furlan R. **Microvesicles: novel biomarkers for neurological disorders.** Front Physiol. 2012 Mar 29;3:63.