Déclarations d'intérêts

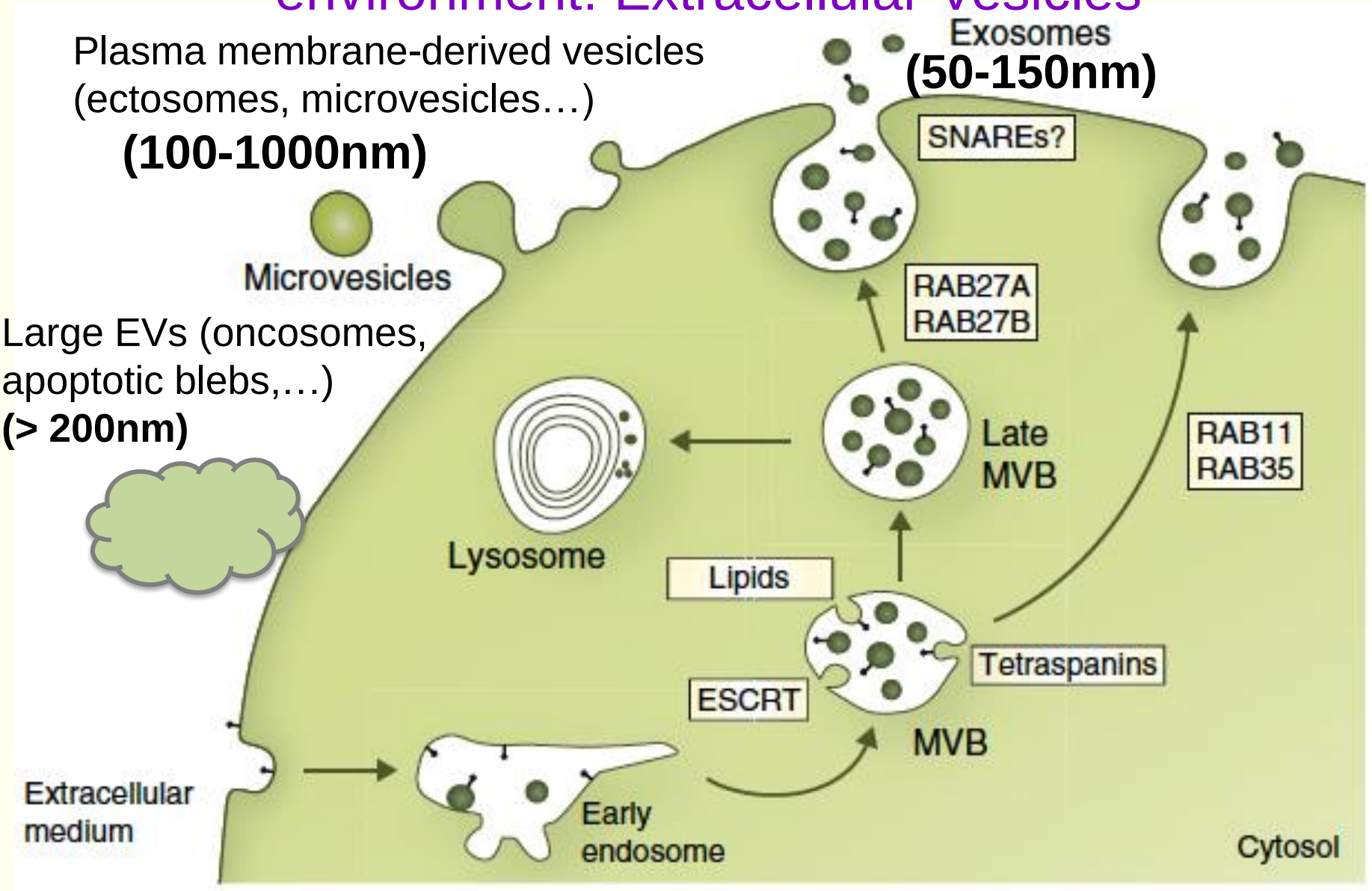
L'objectif de cette déclaration est d'exposer aux congressistes l'existence d'éventuels liens qui pourraient influencer, d'une façon ou d'une autre, votre intervention.

Je déclare ne pas avoir de conflits d'intérêts en rapport avec mon intervention

Exosomes and other Extracellular Vesicles: Definition and roles in communication between tumors and the immune system

Clotilde Théry, PhD
Institut Curie, INSERM U932, « Immunity and Cancer », Paris
No potential COI to disclose

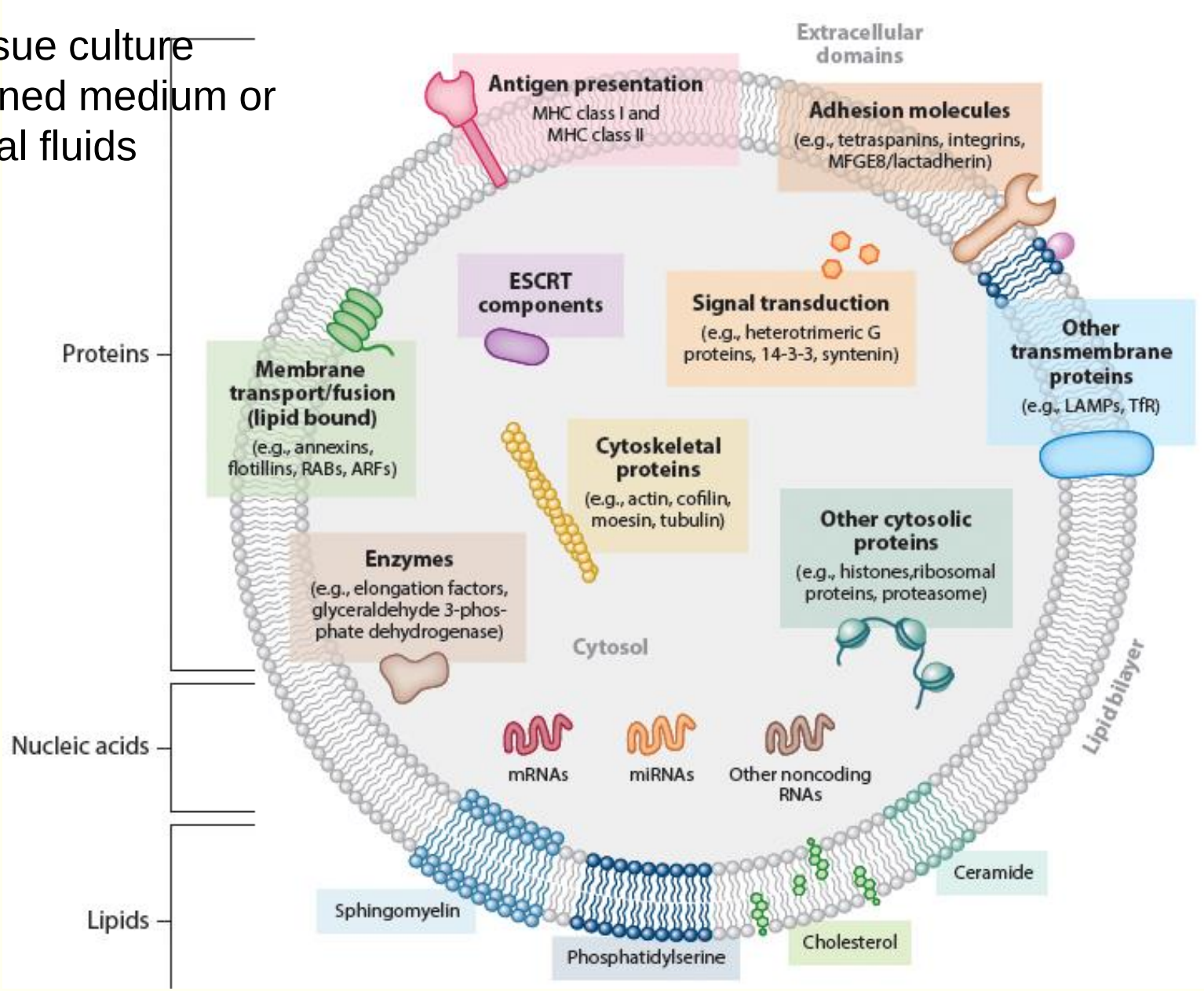
Cells release various types of membrane vesicles in their environment: Extracellular Vesicles



adapted from Kowal, Tkach* and Théry, Curr Op Cell Biol 2014*

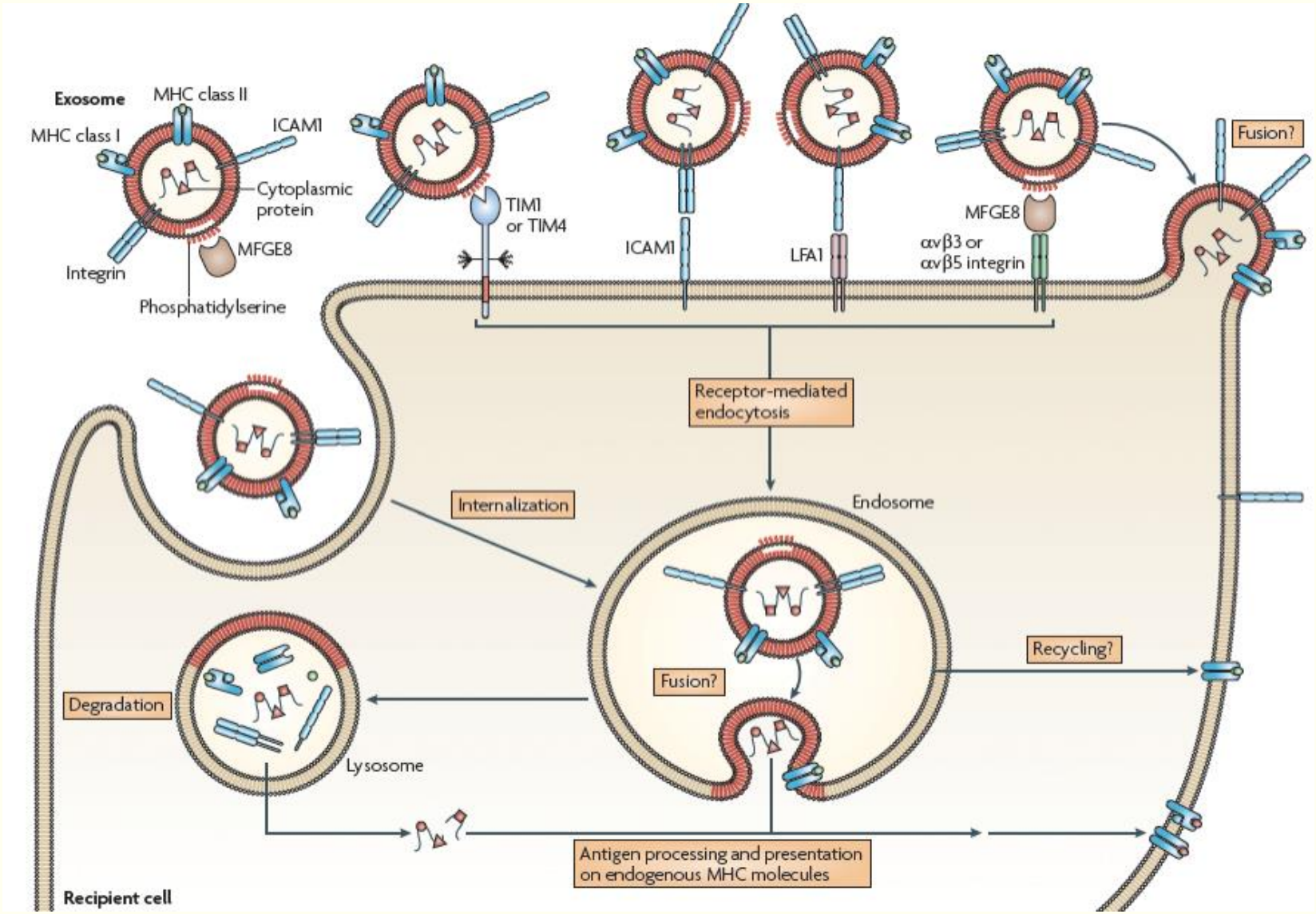
General structure and composition of exosomes/EVs

from tissue culture conditioned medium or biological fluids



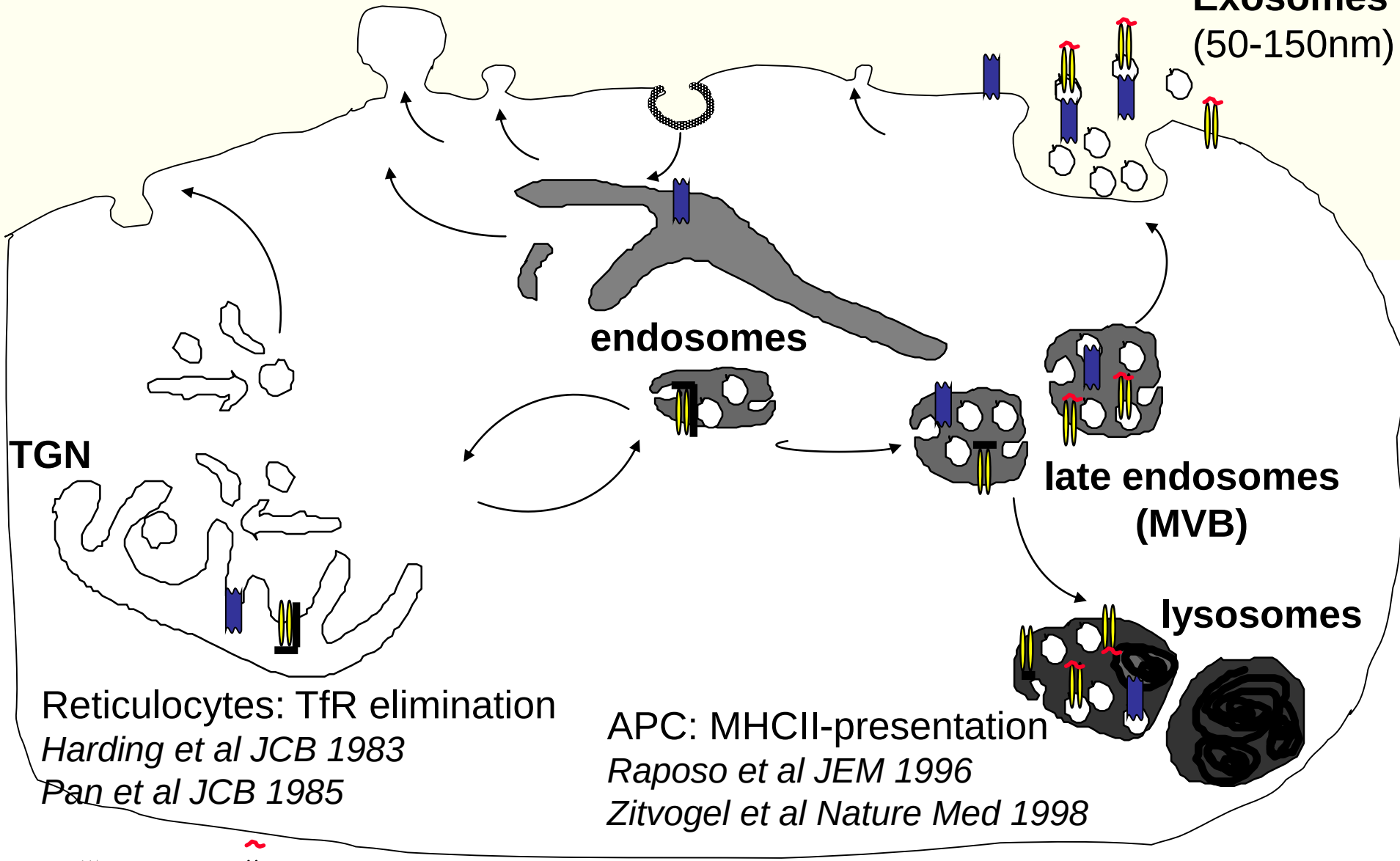
Colombo, Raposo and Théry*, Ann Rev Cell Dev Biol 2014*

Exosomes/EVs can interact in various ways with surrounding cells

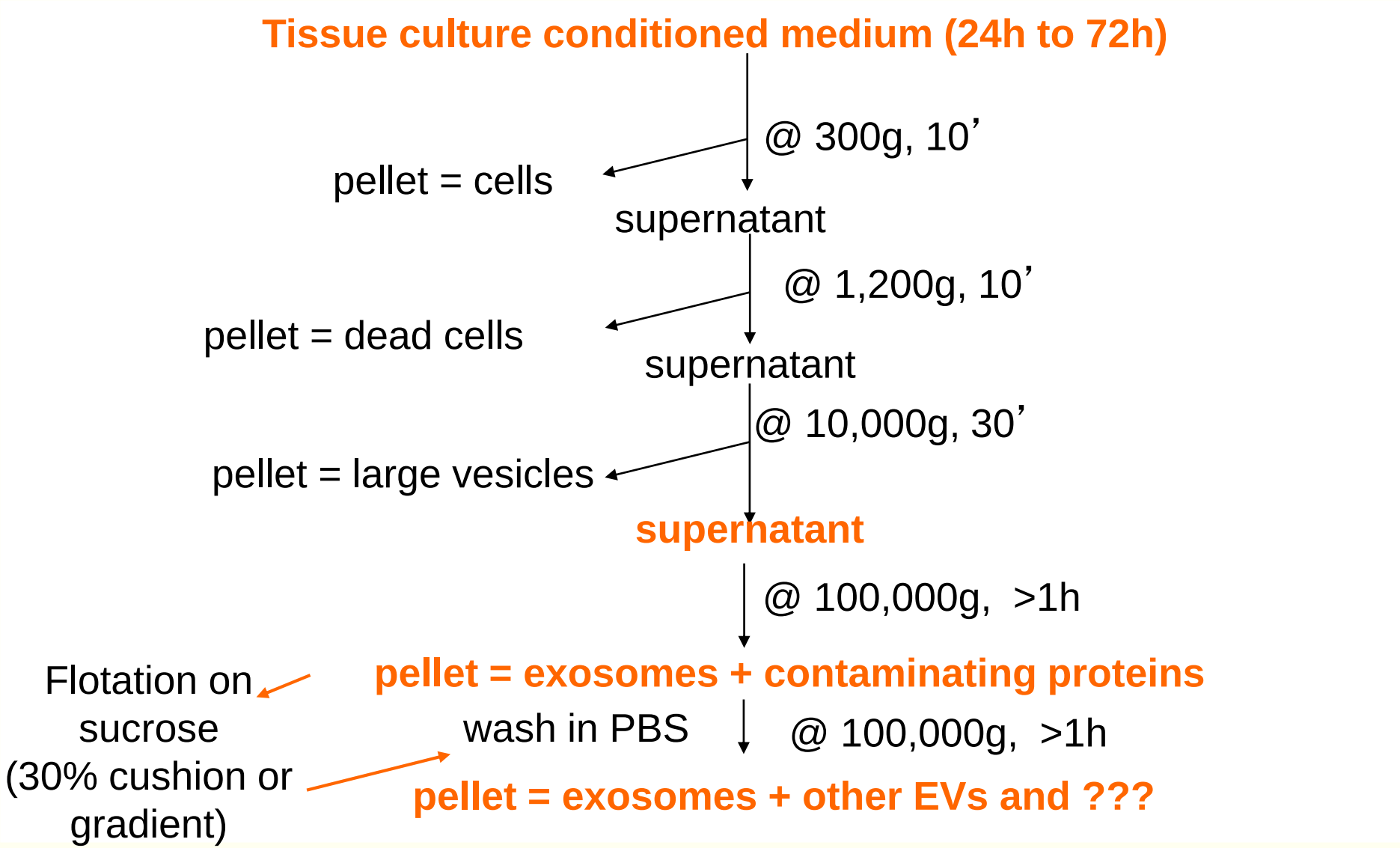


Théry et al, Nature Rev Immunol 2009

exosomes : secreted membrane vesicles originating from endosomal multivesicular compartments



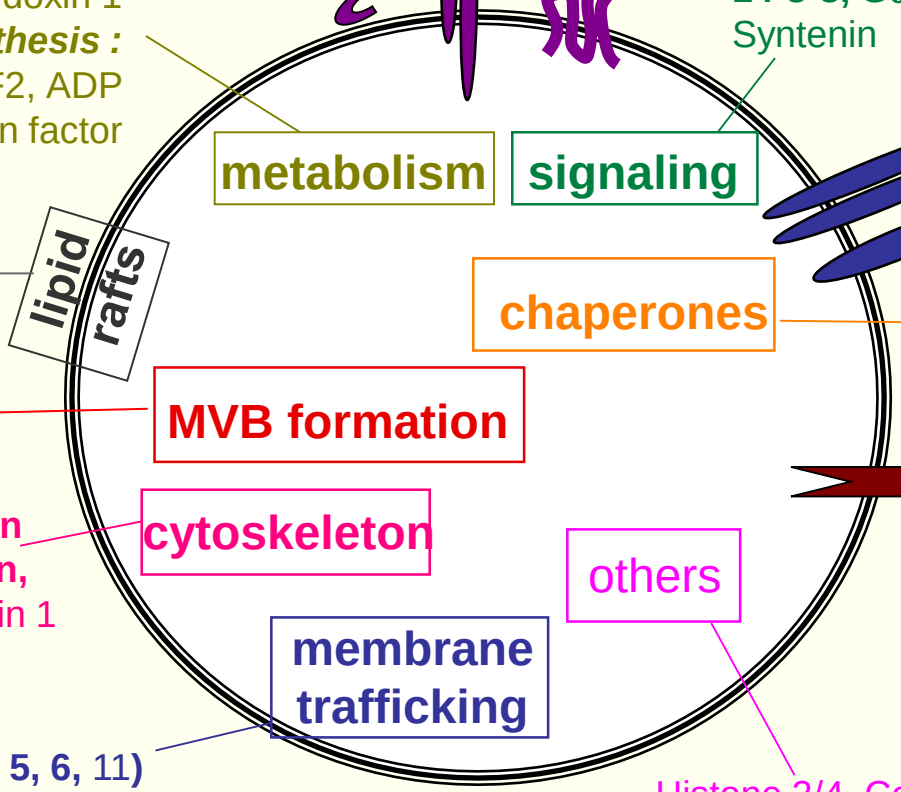
Exosome isolation from **cell culture conditioned medium** by differential ultracentrifugation



Thery et al, Current Protoc Cell Biol 2006

protein composition of a « canonical » exosome

generally purified by
differential ultracentrifugation
(final: 100,000g)



Targeting / adhesion

Integrins

Tetraspanins (CD63, CD81, CD9)

MFGE8

Enzymes:
GAPDH, Pyruvate kinase, αEnolase, Phosphoglycerate kinase 1, Peroxiredoxin 1

Protein synthesis :
Ef1α1, eEF2, ADP ribosylation factor

14-3-3, Gα, Syntenin

T cell stimulating molecules

MHC II
MHC I

metabolism

signaling

Flottilin-1

lipid rafts

chaperones

Hsc70, Hsp90
cyclophilin A

Alix, Tsg101, **clathrin,** Ubiquitin

MVB formation

Transmembrane molecules

Actin, Tubulin
Ezrin, Moesin,
Radixin, Cofilin 1
myosin

cytoskeleton

others

membrane trafficking

CD26/Dipeptidylpeptidase
CD13/Aminopeptidase
ATPase Na+/K+ transporting
CD98

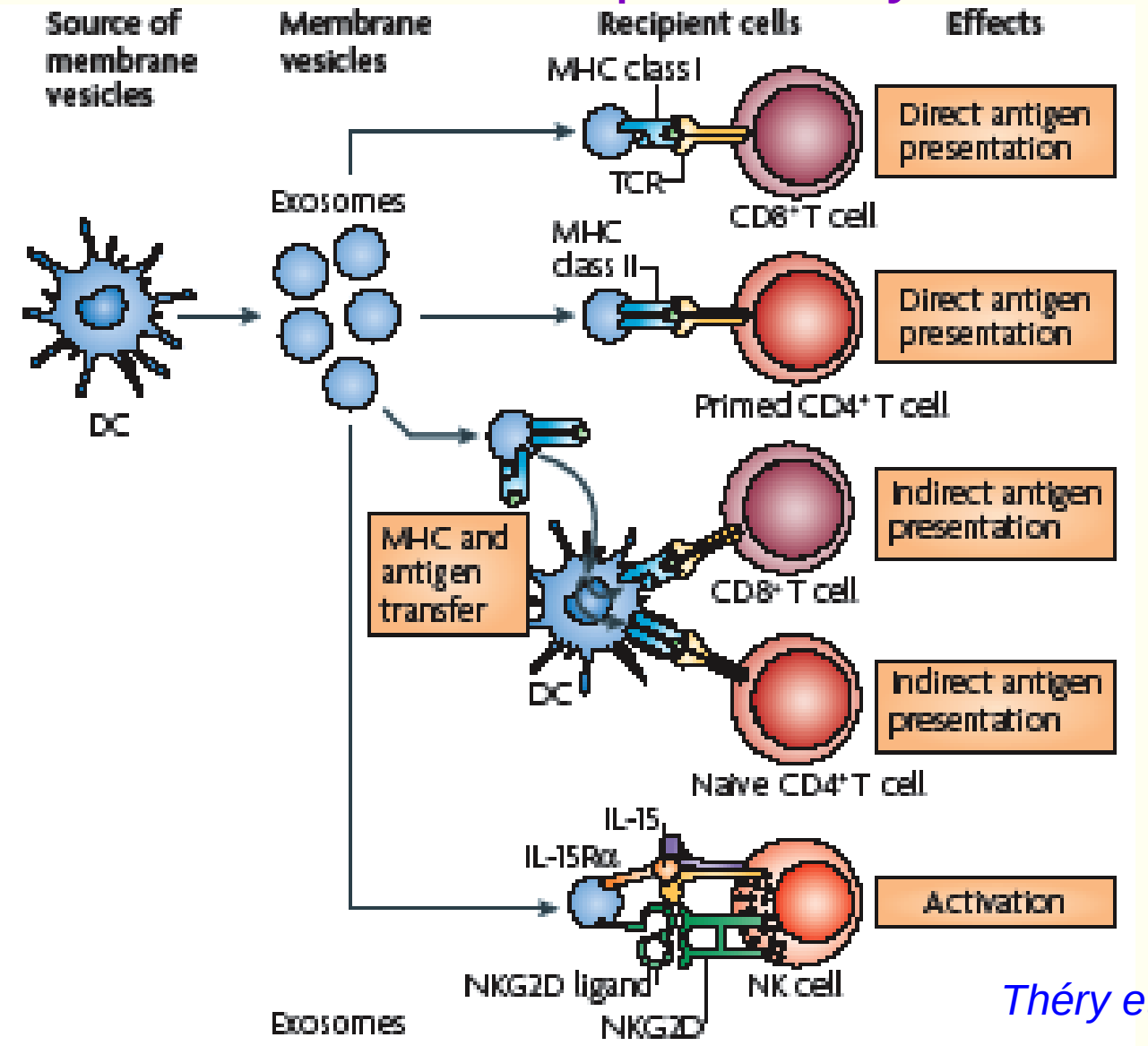
Annexins (1, **2, 4, 5, 6,** 11)
Rab (2, **7,** 10, 11B**),**
Rab GDI, Rap1B

Histone 2/4, Complement factor, MVP, ferritin, atypically secreted proteins

compilation of proteomic studies 1999-2010:
http://microvesicles.org

Chaput and Théry, Semin Immunopathol. 2011

Exosomes secreted by dendritic cells carry MHC-peptide complexes which can activate T cell responses: a means to increase probability of T cell activation



Groups:

Amigorena/Thery (France) (*Nat Immunol 2002, Blood 2005, JI 2007*)

Zitvogel/Chaput (France) (*Nat Med 1998, JI 2004*)

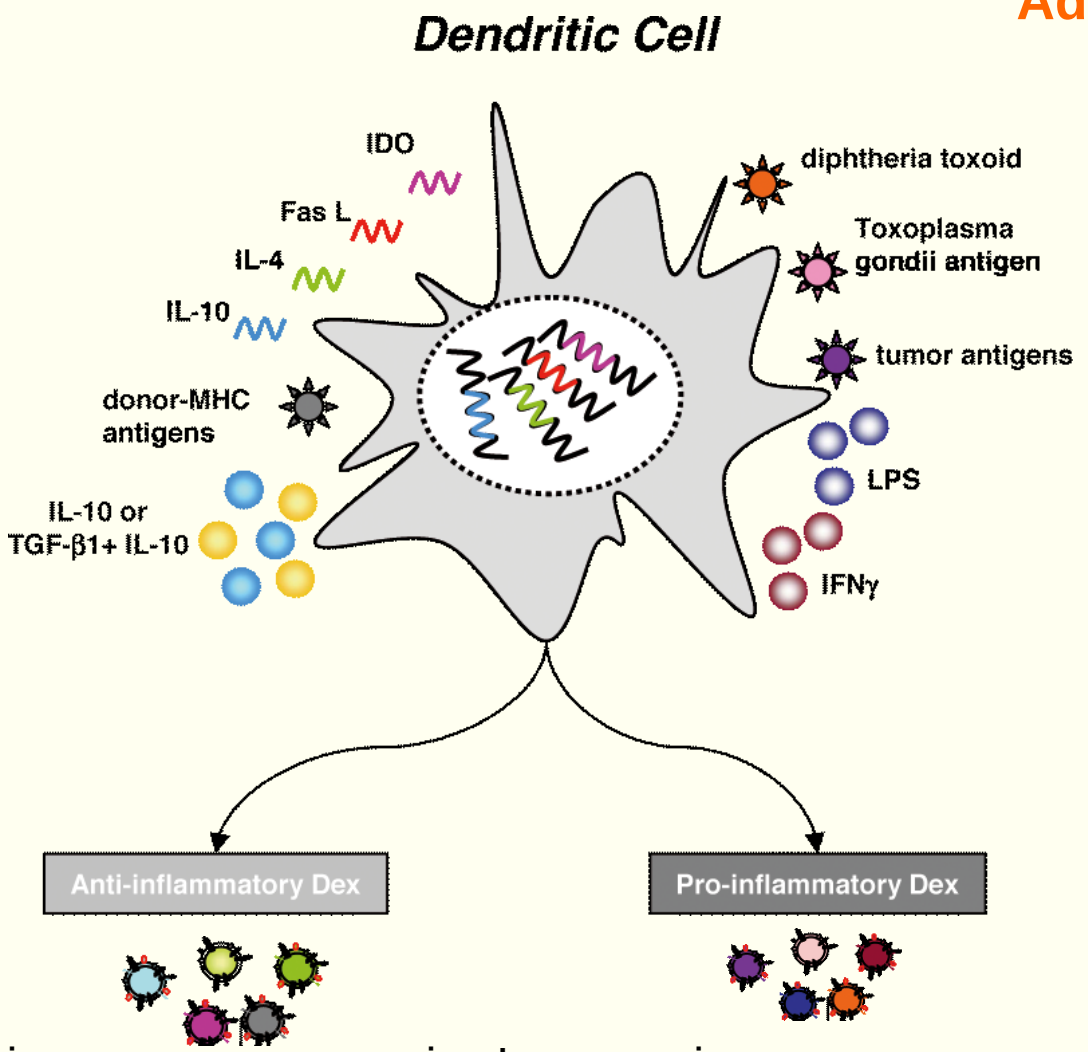
Gabrielsson (Sweden)

Morelli (USA)

Wauben/Stoorvogel (NL)

Théry et al., Nat Rev Immunol. 2009

Possible uses of DC-exosomes in immunotherapy



Decrease immune responses in:
Rheumatoid arthritis
Transplantation
Allergy

Increase immune responses in:
Infection
Cancer

Advantages of exosomes

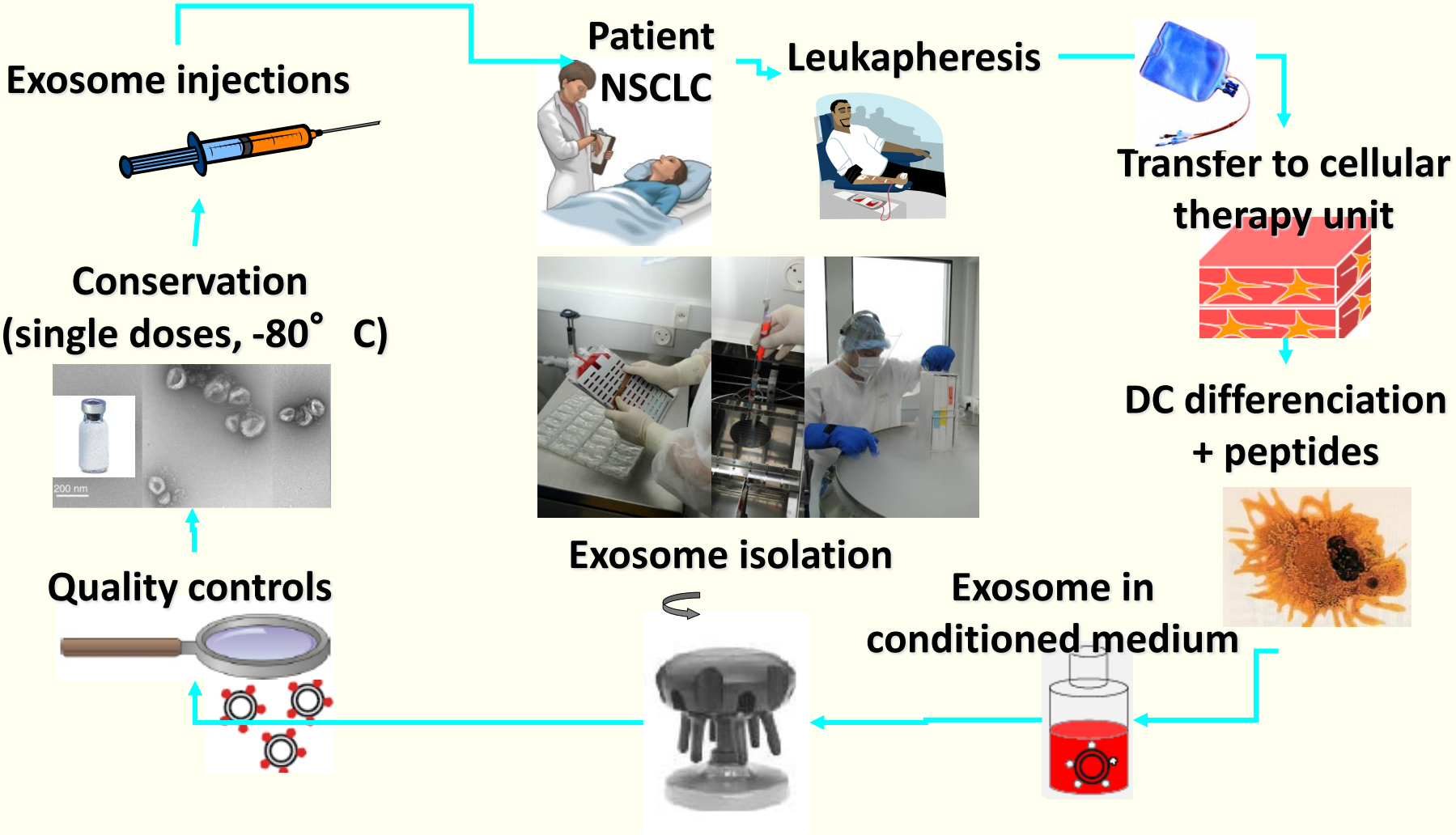
DCs die during freezing: no control on the exact number of injected cells

Properties of DCs can change after injection in vivo: MHC/peptide complexes, cytokines…

Exosomes are nanovesicles stable from freezing to after injection in vivo

Viaud et al., Cancer Res. 2010

2001-2005: Phase I Clinical trials using DC-exosomes as immunotherapy treatment in advanced cancers



Morse et al., J Transl Med 2005; Escudier […] Zitvogel, J. Transl Med 2005 :

feasibility and **safety**

2011-2015: Phase II Clinical trial using IFNγDC-exosomes as immunotherapy treatment in NSC Lung cancer

ONCOIMMUNOLOGY
2016, VOL. 5, NO. 4, e1071008 (13 pages)
http://dx.doi.org/10.1080/2162402X.2015.1071008

Institut de cancérologie GUSTAVE ROUSSY VILLEJUIF - www.igr.fr

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ORIGINAL RESEARCH

L. Zitvogel / N. Chaput

OPEN ACCESS

Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC

Benjamin Besse[a,b,c,*], Mélinda Charrier[a,c,d,e,*], Valérie Lapierre[a,f], Eric Dansin[g], Olivier Lantz[e,h,i], David Planchard[b], Thierry Le Chevalier[b], Alain Livartoski[j], Fabrice Barlesi[k], Agnès Laplanche[l], Stéphanie Ploix[e], Nadège Vimond[d,e], Isabelle Peguillet[e,h,i], Clotilde Théry[c,i], Ludovic Lacroix[m,n], Inka Zoernig[o], Kavita Dhodapkar[p,q], Madhav Dhodapkar[q,r,s], Sophie Viaud[a,c,t], Jean-Charles Soria[a,c,u,v], Katrin S. Reiners[w], Elke Pogge von Strandmann[w], Frédéric Vély[x,y,z,aa], Sylvie Rusakiewicz[a,e,t], Alexander Eggermont[a,c,t], Jonathan M. Pitt[a,c,t], Laurence Zitvogel[a,c,e,t,*], and Nathalie Chaput[a,d,e,f,*]

institutCurie Together, let's beat cancer.

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Association pour la Recherche sur le Cancer

Instituts thématiques Inserm Institut national de la santé et de la recherche médicale

Institut National du Cancer

Cancéropôle Île-de-France

îledeFrance

22 patients included, 1 with grade 3 hepatotoxicity

Primary endpoint = 50% patients with progression-free survival >4 months:

not reached (32% patients with PFS> 4 months)

No clear T cell responses

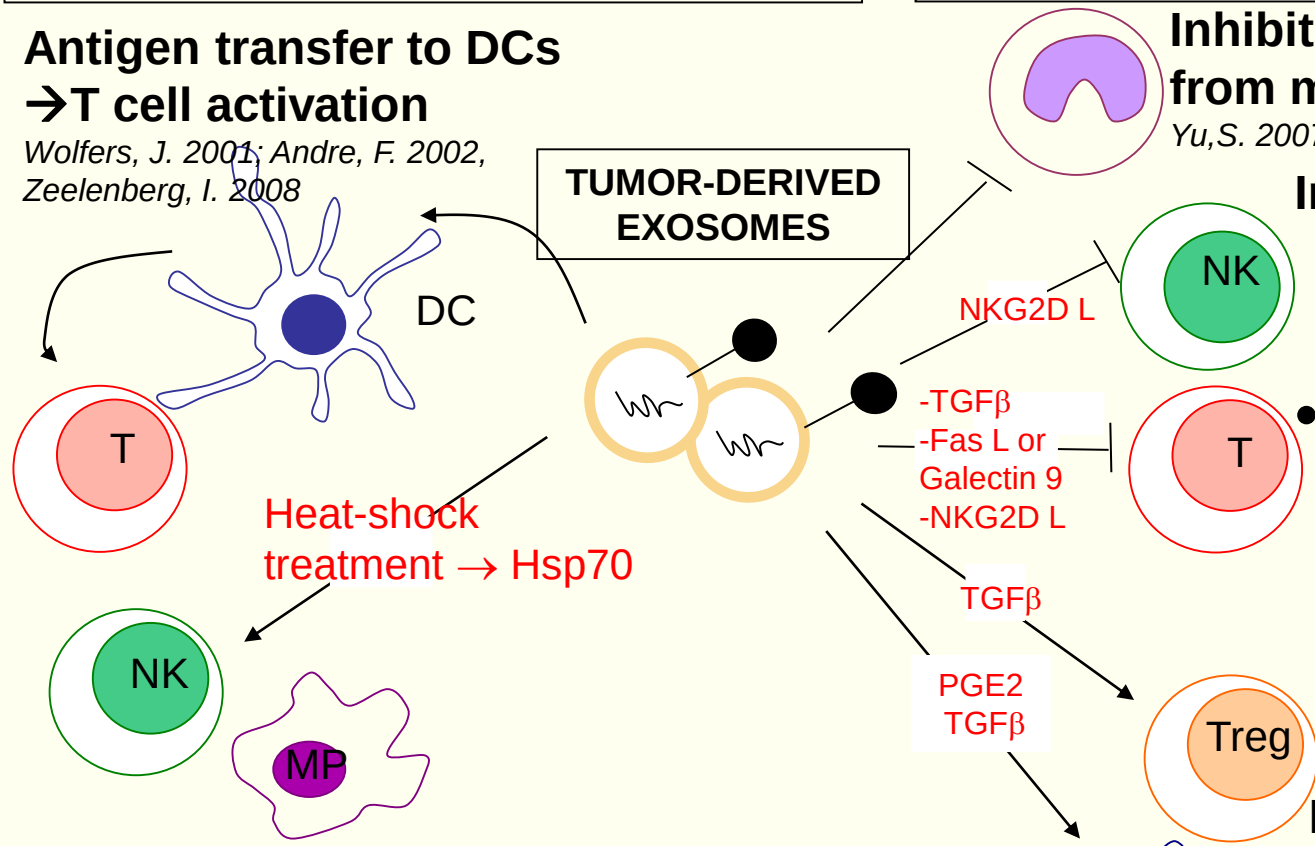
Increase of NK cell activity in some patients with initial decrease of NKp30 expression, correlated with longer progression-free survival.

Tumors secrete exosomes with various (contradictory) effects on the immune system

ANTI-TUMORAL: stimulate anti-tumor immunity

PRO-TUMORAL: inhibit anti-tumor immunity

Antigen transfer to DCs
→T cell activation
Wolfers, J. 2001; Andre, F. 2002, Zeelenberg, I. 2008



Activation of NK cells and macrophages
Vega, V.L. 2008;
Gastpar, R. 2005

Inhibition of DC differentiation from myeloid precursors,
Yu,S. 2007

Inhibition of NK cells cytotoxic activity
Liu,C. 2006; Clayton,A. 2008

Inhibition of T cells :
- **decreased proliferation**

Clayton,A. 2007
- **T cell killing**

Andreola, G. 2002; Huber, V. 2005
- **decreased cytotoxic activity**

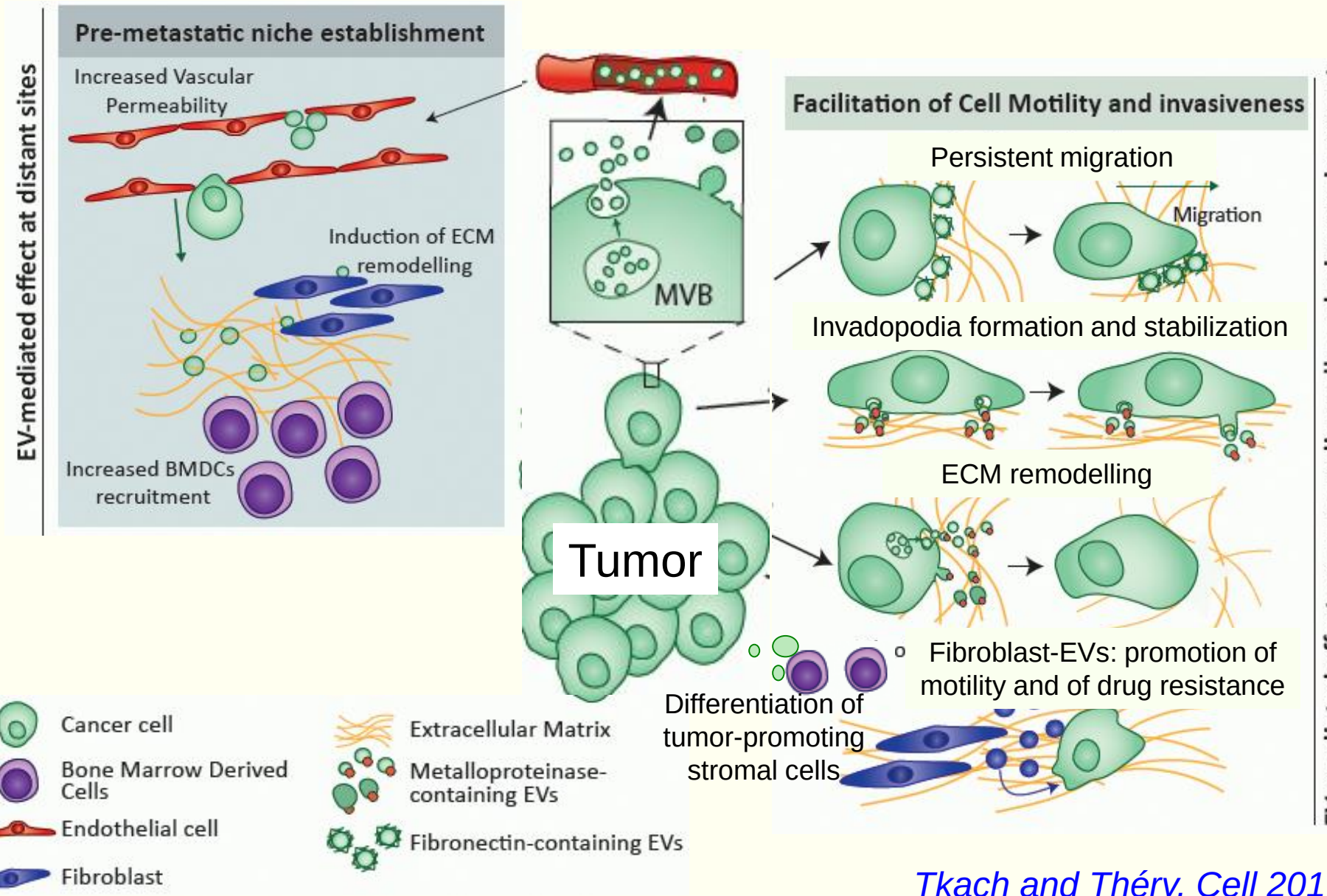
Clayton,A. 2008; Klibi, J. 2009

Promotion of Treg
Clayton,A. 2007

Promotion of MDSCs
Xiang, X 2009; Chalmin F. 2010

Bobrie et al, Traffic 2011

Tumor-derived exosomes/EV act locally on cells of the tumor microenvironment, and at a distance for metastasis



Tkach and Théry, Cell 2016

Concept arising from in vitro studies:

exosomes/EVs= **a new complex mode of cell-to-cell communication** carrying proteins, nucleic acids, lipids

Present in biological fluids (blood, urine, milk…): thus **they exist in vivo**

Involved in all possible pathological and physiological systems (immune responses, cancer but also cardiovascular, nervous…)

But many remaining unknowns:

Are exosomes **more, or less, or equally** functionally relevant than other EVs?

Is EV-mediated transfer of functional miRNA and/or mRNA really happening in physiological conditions ?

Why don't we know the respective functional importance of exosomes and other EVs?

Cell

Leading Edge
Review

Communication by Extracellular Vesicles: Where We Are and Where We Need to Go

Mercedes Tkach[1] and Clotilde Théry[1,*]
[1]Institut Curie, PSL Research University, INSERM U932, 75005 Paris, France

Cell *164*, March 10, 2016

EV research is now at the stage where the immunology field was in the 1950s.

At that time, researchers could claim that circulating **white blood cells** were capable of very different functions, such as **killing other cells** or **making antibodies**, simply because there were no means to distinguish what **we know now as B versus T lymphocytes**!

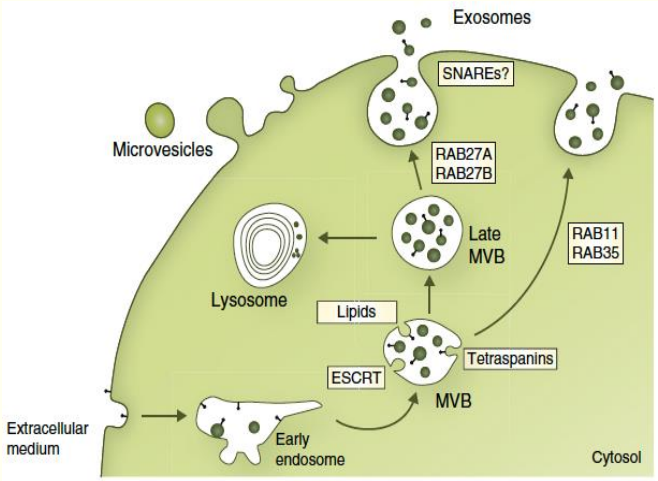
Today, we are still analyzing the functions of exosomes and/or EVs as mixtures of heterogeneous vesicles

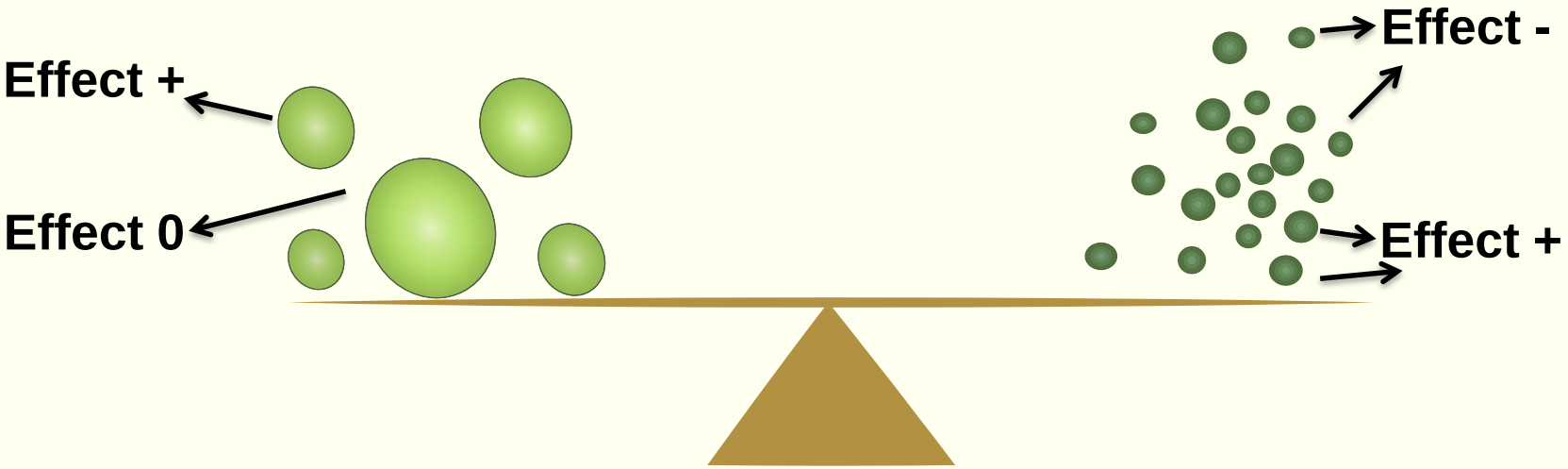
Discrepancies in proposed functions of tumor- or DC-derived exosomes could be due to different natures or heterogeneities of the analyzed « exosomes »

Different sub-cellular origin => different functions?

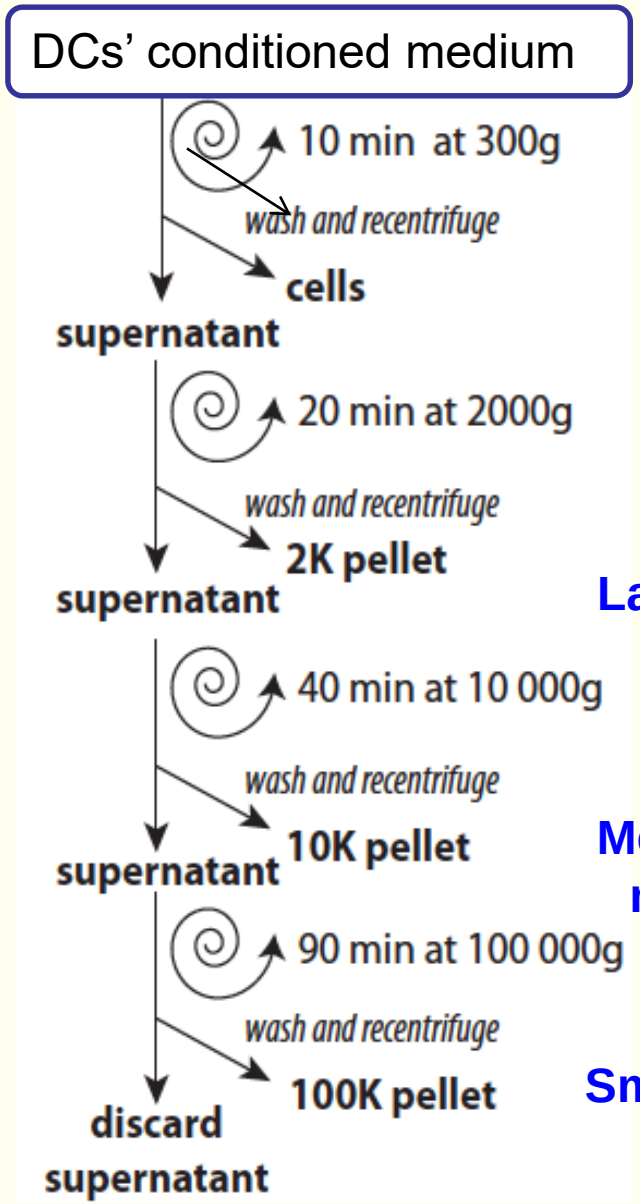
Different sub-cellular origins should be reflected in different protein compositions

Need tools to separate and distinguish subpopulations of EVs





Framework to establish the respective characteristics of different EV subtypes: comparative analysis after (partial) separation

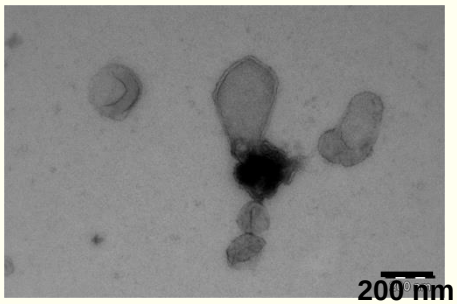


2K pellet
Large/heavy EVs

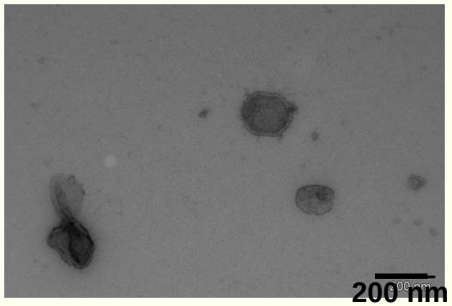
10K pellet
Medium EVs (e.g. microvesicles)

100K pellet
Small size EVs (e.g. exosomes)

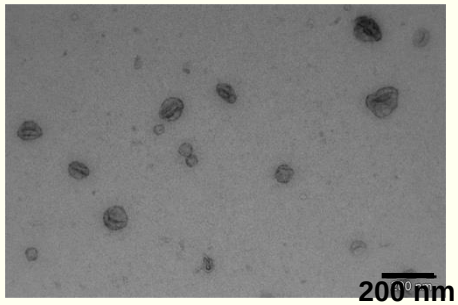
2K

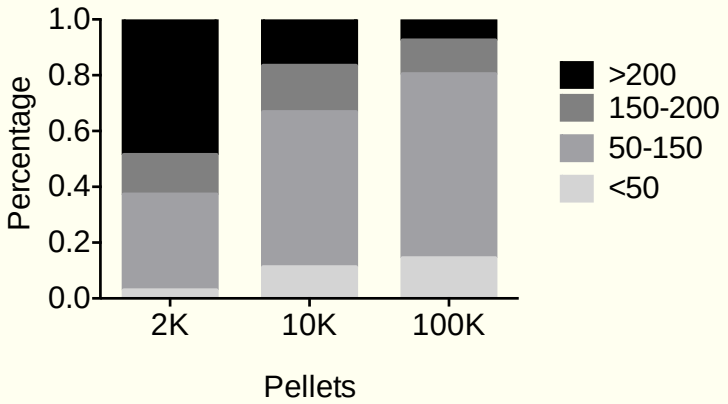


10K



100K

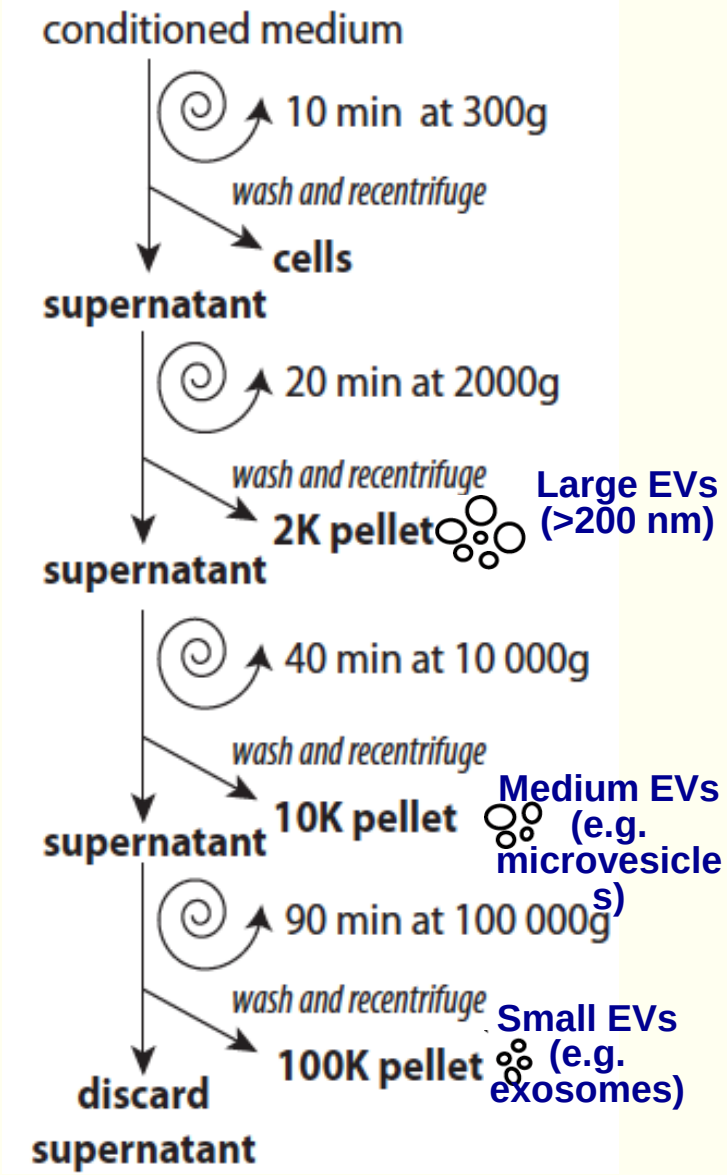




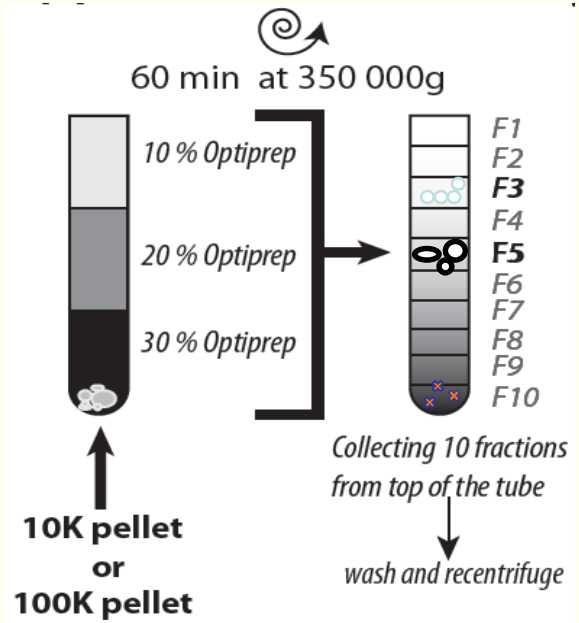
Kowal et al. PNAS 2016

Various means to separate different EV subtypes

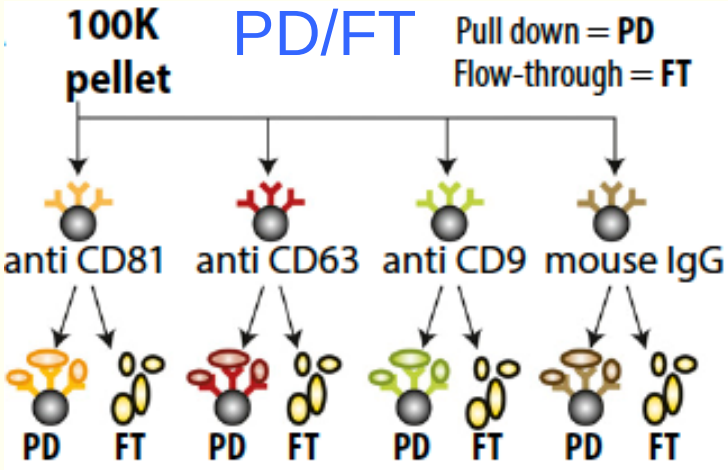
1- Differential centrifugation



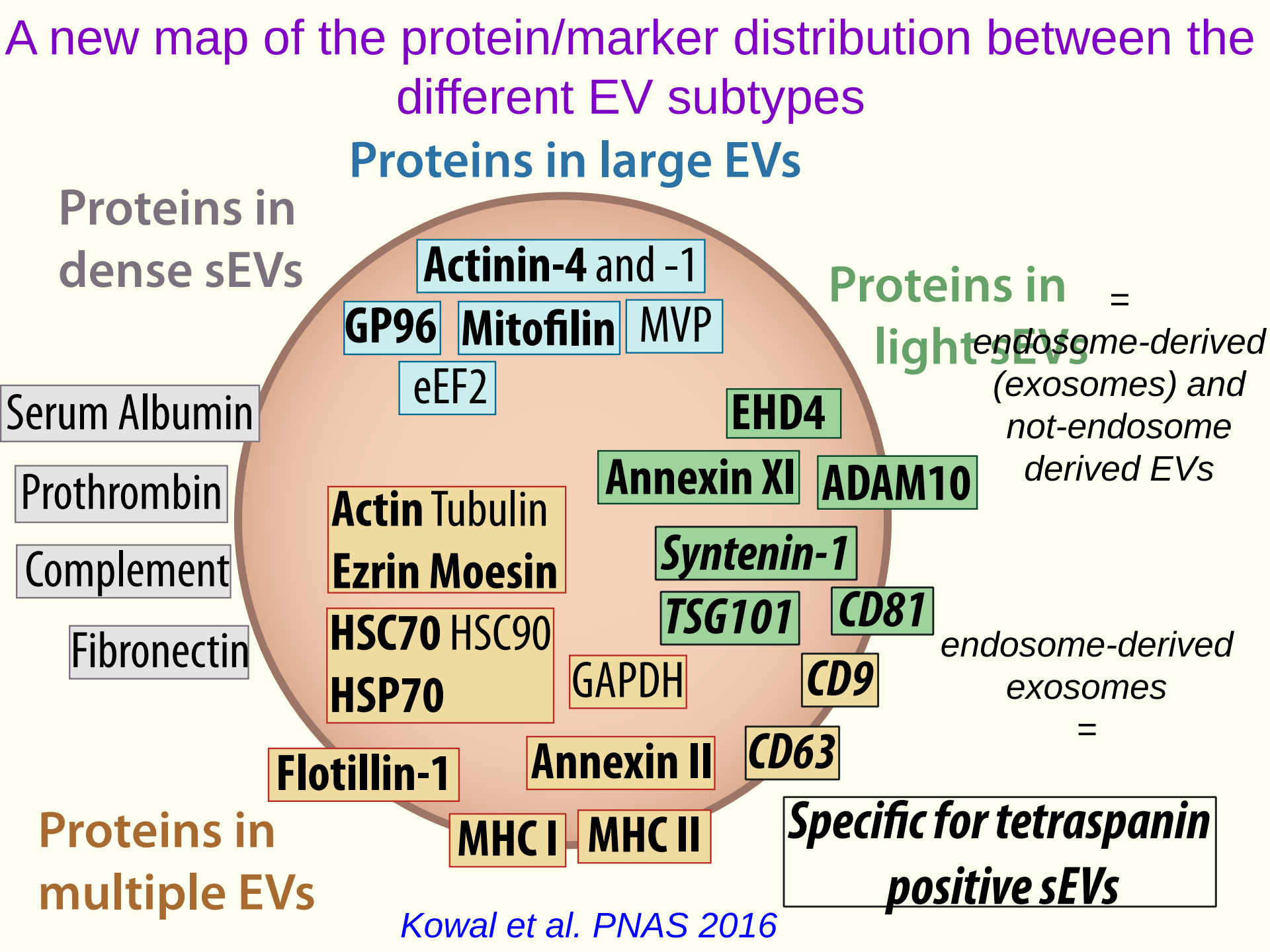
2a- Floatation into density gradient



2b-Immuno-isolation and comparison



A new map of the protein/marker distribution between the different EV subtypes



Kowal et al. PNAS 2016

Remaining questions

➡ what are the specific (e.g. immune) functions of the different subtypes of EVs? Can we improve their use for therapeutic purposes: cancer immunotherapy?

➡ what is the diagnostic/prognostic value of the different subtypes of EVs in biofluids?

Glypican-1 identifies cancer exosomes and detects early pancreatic cancer

Sonia A. Melo[1]†*, Linda B. Luecke[1]*, Christoph Kahlert[1]*, Agustin F. Fernandez[2], Seth T. Gammon[3], Judith Kaye[1], Valerie S. LeBleu[1], Elizabeth A. Mittendorf[4], Juergen Weitz[5], Nuh Rahbari[5], Christoph Reissfelder[5], Christian Pilarsky[5], Mario F. Fraga[2,6], David Piwnica-Worms[3] & Raghu Kalluri[1]

9 JULY 2015 | VOL 523 | NATURE | 177

R. Kalluri

GPC1 level measured in total plasma EVs (possible problem with antibody), prognostic value in pancreas, but not breast cancer

➡ Is *in vivo* secretion of exosomes and/or other EVs really playing any patho- or physiological role?

Approaches of inhibition of EV secretion (e.g. shRNA against Rab proteins), or of transfer in vivo of enzyme or RNA: EV specificity to be now determined

Any EV study must

explore the heterogeneity of the EV population analyzed

demonstrate association of the function/feature attributed to EV by specific co-isolation

Journal of Extracellular Vesicles

COACTION

EDITORIAL

Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles

« MISEV » Minimal Informations for Studies on EVs

Secreted membrane-enclosed vesicles, collectively called extracellular vesicles (EVs), which include exosomes, ectosomes, microvesicles, microparticles, apoptotic bodies and other EV subsets, encompass a very rapidly growing scientific field in biology and medicine. Importantly, it is currently technically challenging to obtain a totally pure EV fraction free from non-vesicular components for functional studies, and therefore there is a need to establish guidelines for analyses of these vesicles and reporting of scientific studies on EV biology. Here, the International Society for Extracellular Vesicles (ISEV) provides researchers with a minimal set of biochemical, biophysical and functional standards that should be used to attribute any specific biological cargo or functions to EVs.

Keywords: *extracellular vesicles; microvesicles; microparticles; exosomes; ectosomes; extracellular RNA*

Citation: Journal of Extracellular Vesicles 2014, **3**: 26913 -

INTERNATIONAL SOCIETY FOR EXTRACELLULAR VESICLES

www.isev.org

Annual Meeting – ISEV2017
Toronto, Canada
18-21 May 2017

Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale

COST Action BM1202 ME-HaD
European Network on Microvesicles and Exosomes in Health and Disease

DC BIOL

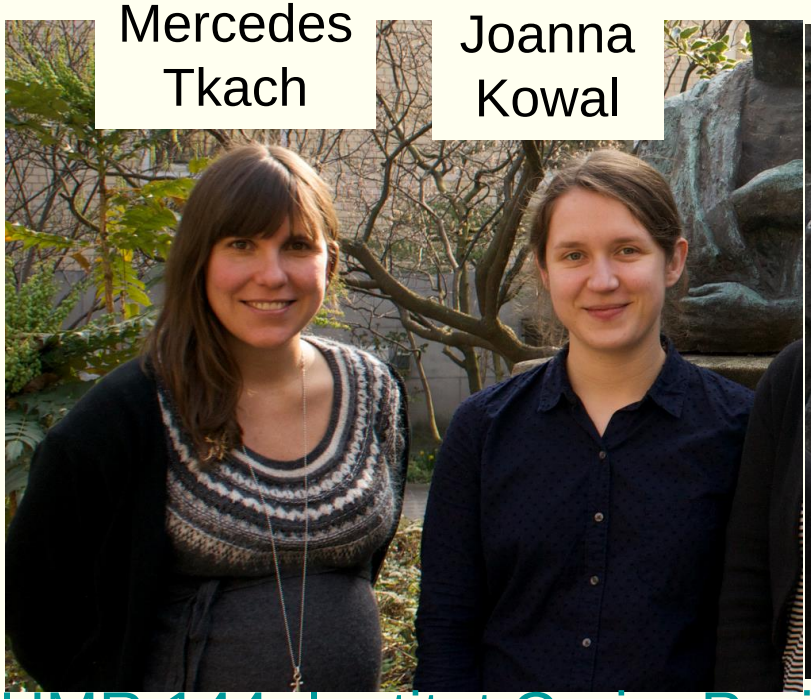
ANR

Association pour la Recherche sur le Cancer

institutCurie
Ensemble, prenons le cancer de vitesse.

INSERM U932, Institut Curie, Paris

"Exosomes and tumor growth"



CNRS UMR 144, Institut Curie, Paris: Graça RAPOSO, G Van NIEL

Proteomics, Institut Curie, Paris: Guillaume ARRAS, Damarys LOEW

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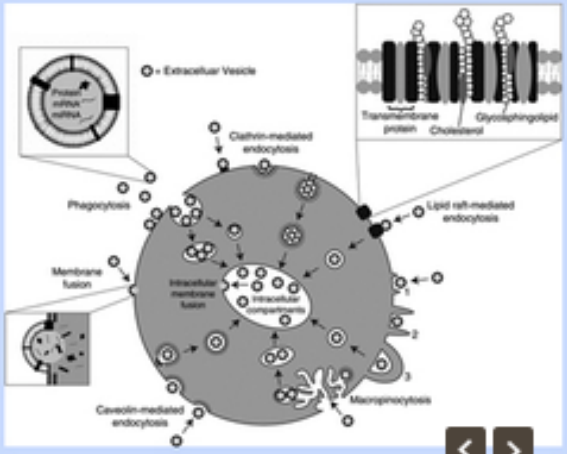
FEATURED: REVIEW ARTICLE

Routes and Mechanisms of Extracellular Vesicle Uptake



About the Journal

Journal of Extracellular Vesicles is a peer-reviewed, open access journal. As the official journal of the International Society for Extracellular Vesicles (ISEV) and ISEV-Americas, it provides a much-needed forum for the exchange of data, ideas and information from all areas pertaining to the chemistry, biology and/or applications of extracellular vesicles, including microvesicles, exosomes, ectosomes, apoptotic bodies and other extracellular vesicles.

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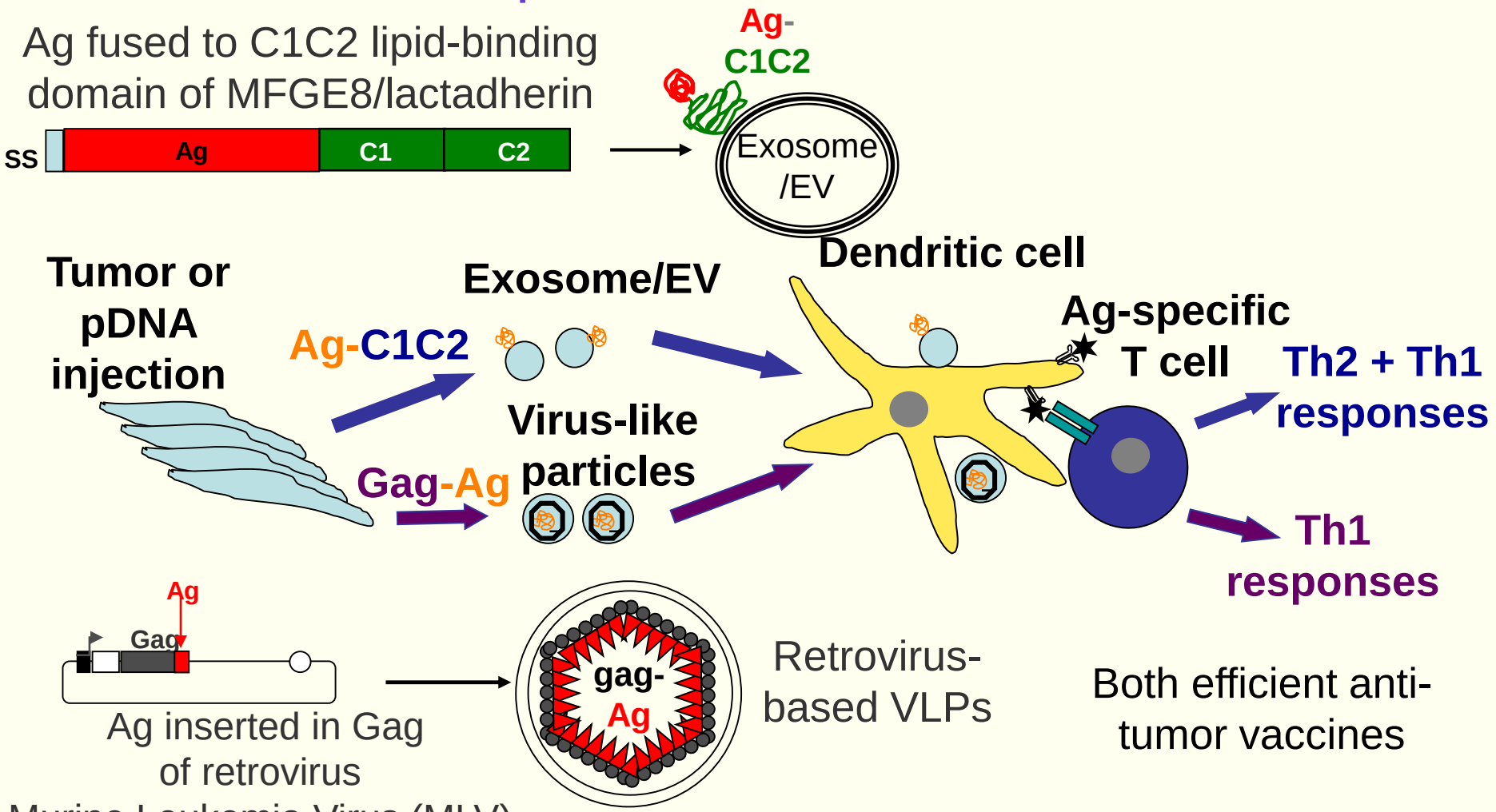
Fusion of an antigen to EV/exosome-targeting sequences allows in vivo induction of antigen-specific immune responses with anti-tumor effect



Zeelenberg et al., Cancer Res. 2008
Sedlik et al, J Extracell Vesicles 2014