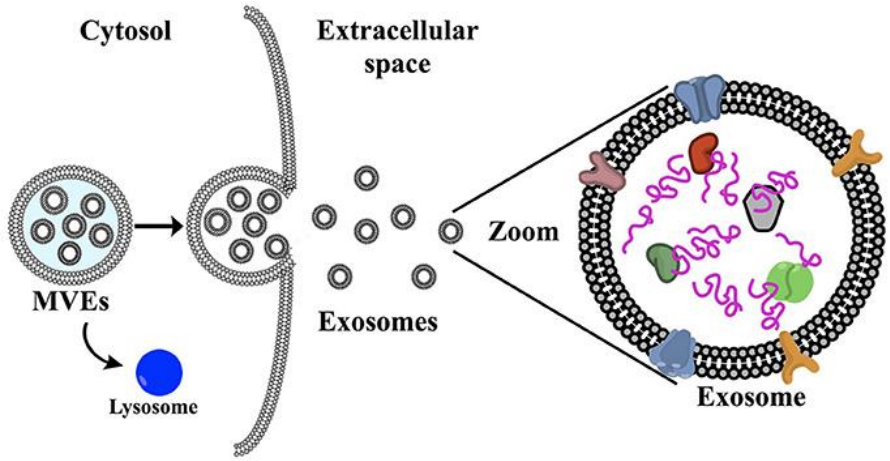


Eksozomlar

Prof. Dr. Tülay Kılıçaslan Ayna

İzmir Katip Çelebi Üniversitesi Tıp Fakültesi Tıbbi Biyoloji AD.

TEAH Doku Tipleme Lab.

Pathology (February 2024) 56(1), pp. 1–10

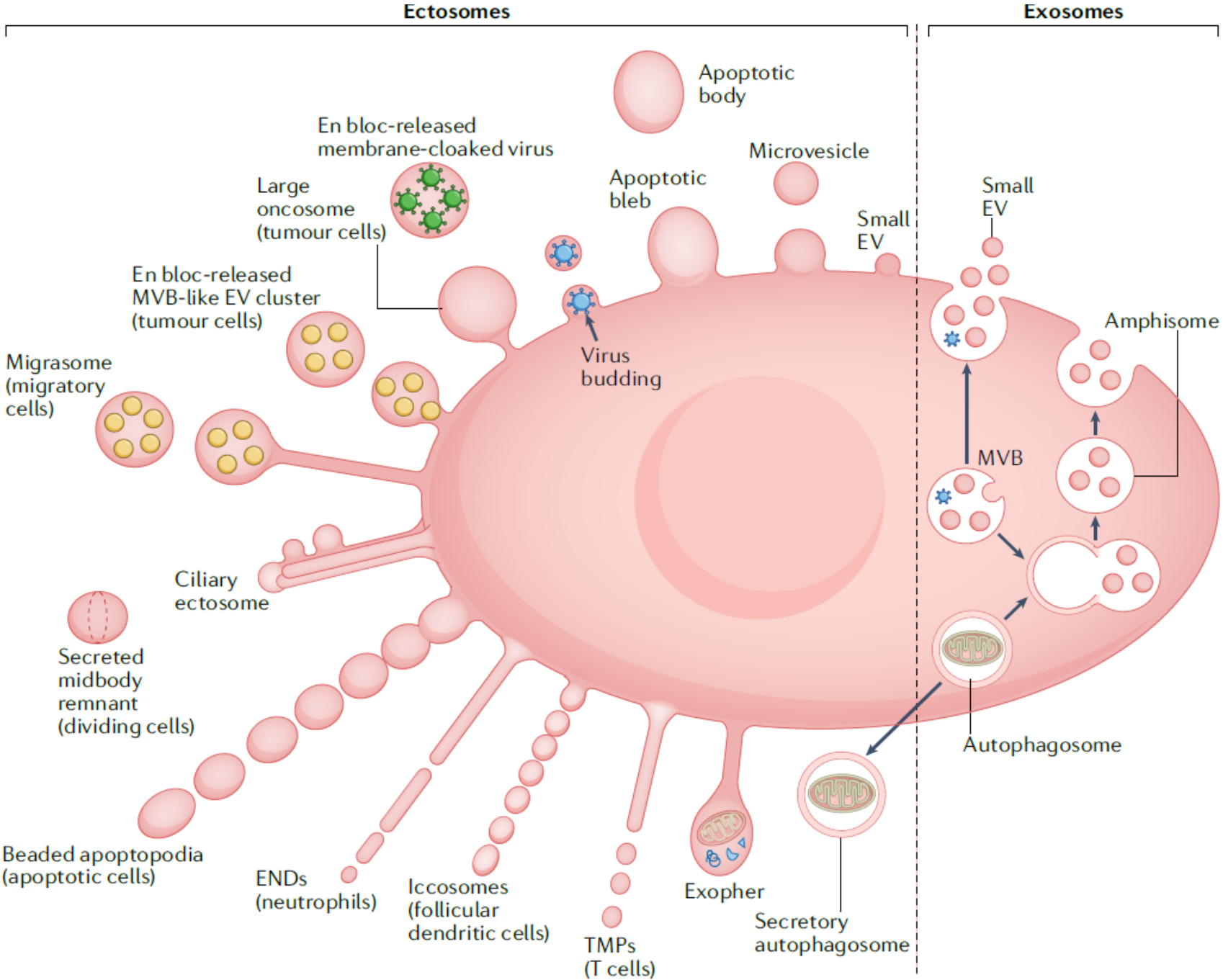
REVIEW

Exosomes and microvesicles in kidney transplantation: the long road from trash to gold

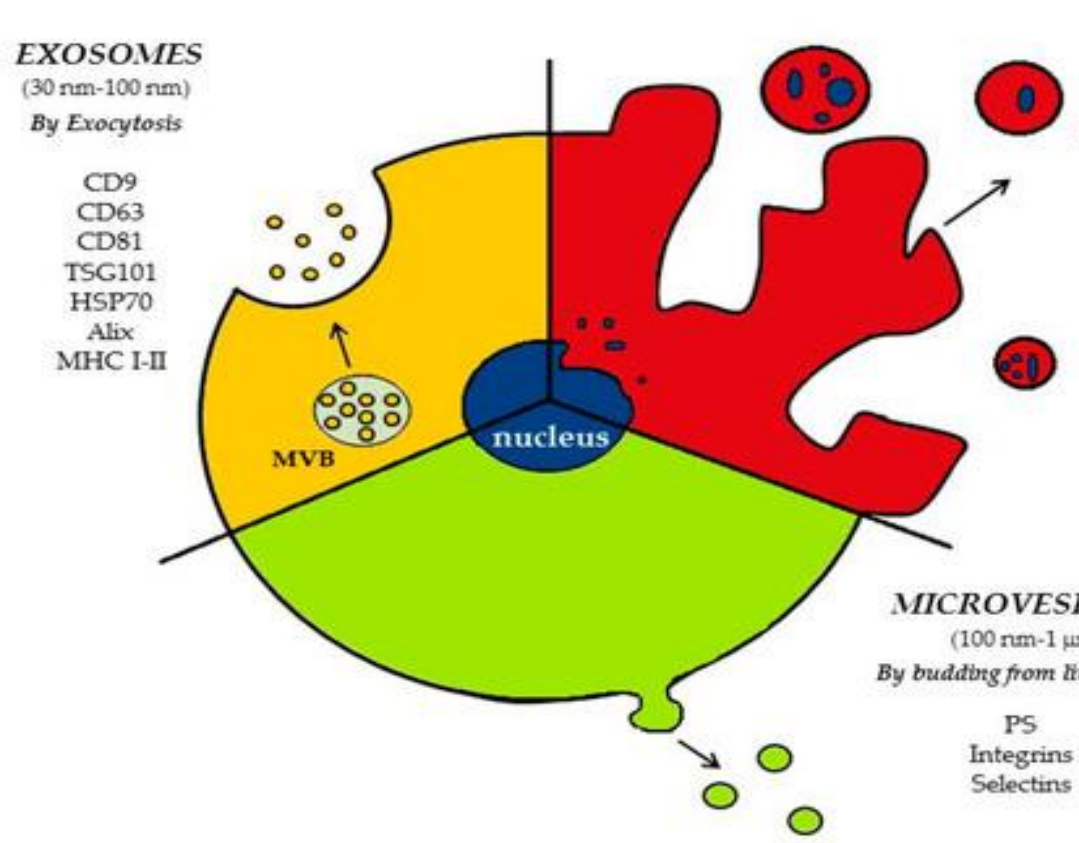
Luis Ramalhete[1,2,3], Ruben Araújo[2], Aníbal Ferreira[2,4], Cecília R. C. Calado[5,6]

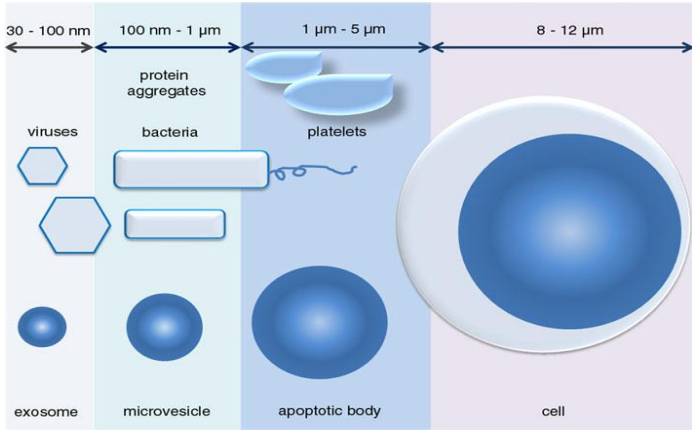
Yıllar	Yayın sayısı
1991-2014	43
EV-2024	4455
Exo-2024	3213

Protein	41860
RNA	7540
miRNA	764
Lipit	1116


Ectosomes
Exosomes
Apoptotic body
En bloc-released membrane-cloaked virus
Microvesicle
Large oncosome (tumour cells)
Apoptotic bleb
Small EV
Small EV
En bloc-released MVB-like EV cluster (tumour cells)
Amphisome
Virus budding
Migrasome (migratory cells)
MVB
Ciliary ectosome
Secreted midbody remnant (dividing cells)
Autophagosome
Beaded apoptopodia (apoptotic cells)
ENDs (neutrophils)
Iccosomes (follicular dendritic cells)
TMPs (T cells)
Exopher
Secretory autophagosome

Uluslararası Extrasellüler veziküller Derneğinin (ISEV) yönergesi;





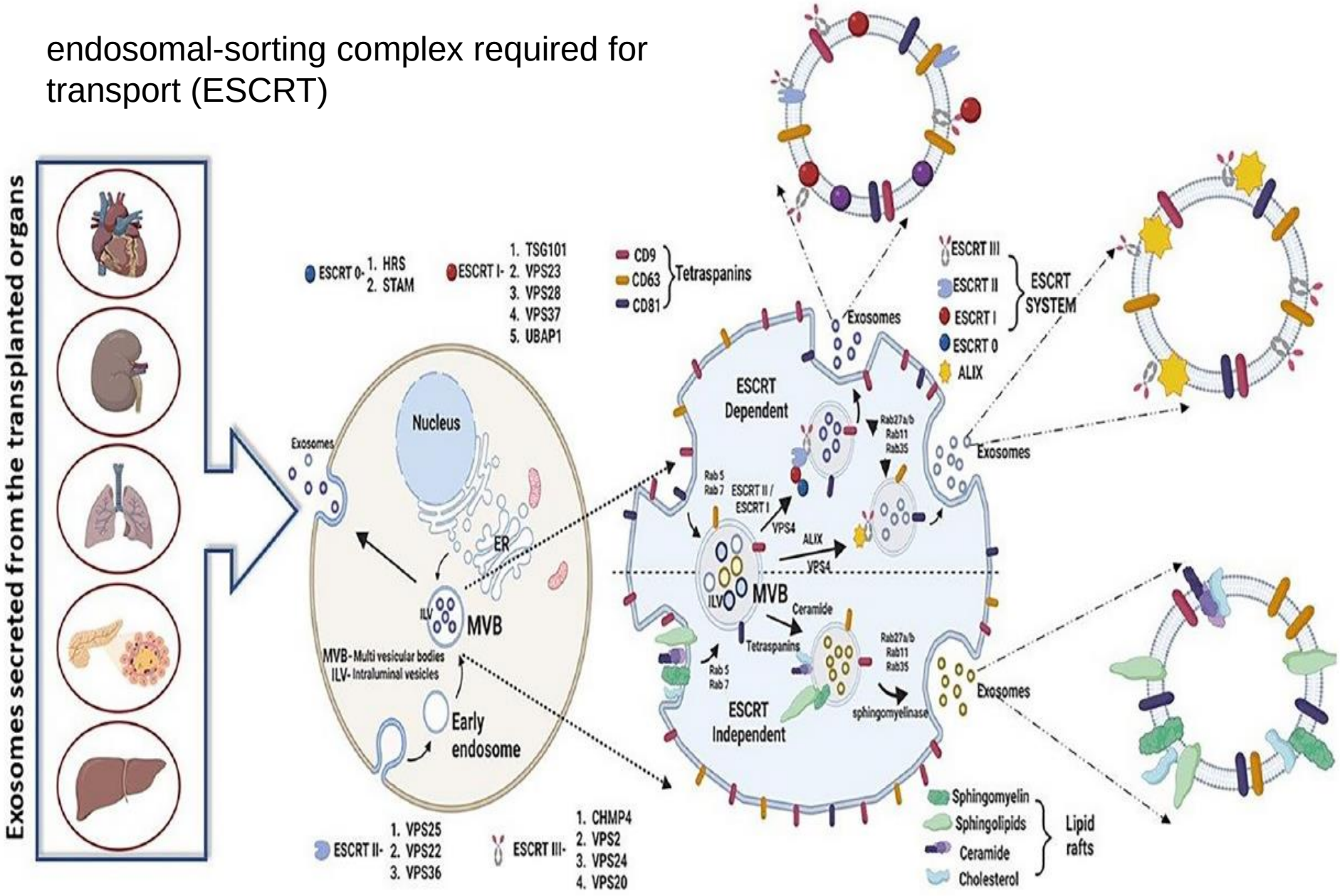
EV'lerin biyokimyasal bileşimi farklıdır; ancak ISEV, EV'lerin izolasyonu, karakterizasyonu ve onayı için yönergeler tanımlamıştır.

Table 1 Main characteristics of exosomes, microvesicles, and apoptotic bodies

	Exosomes	Microvesicles	Apoptotic bodies	References
Size	30–100 nm	100–1,000 nm	1–5 μm	[3]
Origin	Intraluminal vesicles within multivesicular bodies	Plasma membrane and cellular content	Plasma membrane, cellular fragments	[4]
Mechanism of formation	Fusion of multivesicular bodies with the plasma membrane	Outward blebbing of the plasma membrane	Cell shrinkage and programmed cell death	[5, 6]
Release	Constitutive and/or cellular activation	Constitutive and/or cellular activation	Apoptosis	[4]
Time of release	Ten minutes or more	Few seconds	–	[7, 8]
Pathways	ESCRT-dependent Tetraspanin-dependent Ceramide-dependent Stimuli-dependent	Ca^{2+}-dependent Stimuli- and cell-dependent	Apoptosis-related	[3]
Lipid membrane composition	Enriched in cholesterol and ceramide, expose phosphatidylserine, contain lipid rafts	Expose phosphatidylserine, enriched in cholesterol and diacylglycerol, contain lipid rafts	–	[3, 9]
Content	Proteins, mRNA, miRNA, lipids	Proteins, mRNA, miRNA, lipids	Cell organelles, proteins, nuclear fractions, DNA, coding and noncoding RNA, lipids	[3]

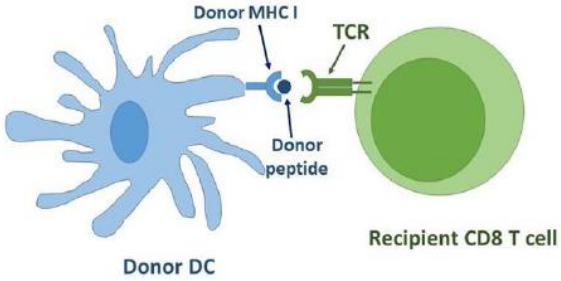
ESCRT endosomal sorting complex required for transport

endosomal-sorting complex required for transport (ESCRT)

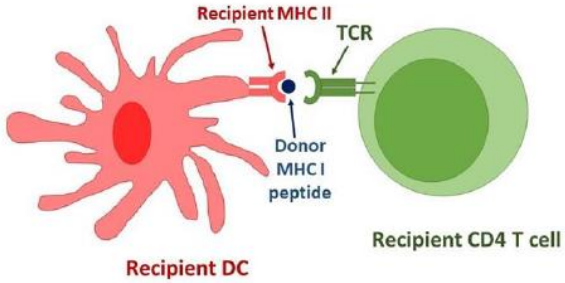


Exosomes in transplantation: Role in allograft rejection, diagnostic biomarker, and therapeutic potential -2023

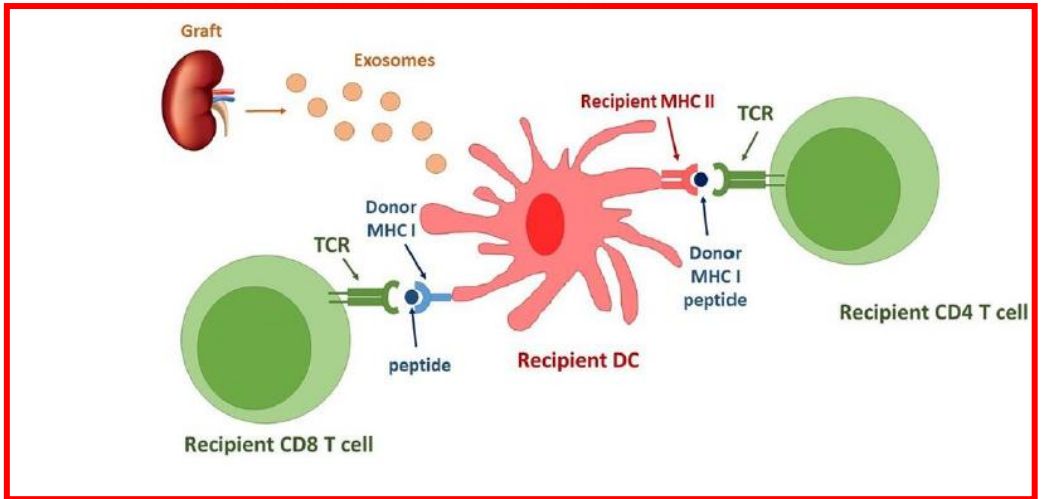
A. DIRECT PATHWAY OF ALLORECOGNITION



B. INDIRECT PATHWAY OF ALLORECOGNITION

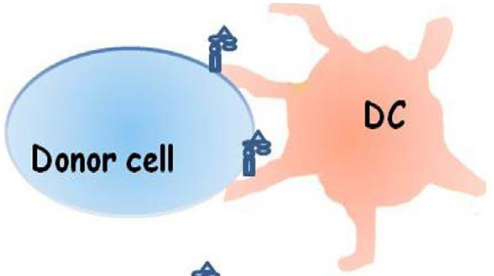


C. SEMI-DIRECT PATHWAY OF ALLORECOGNITION

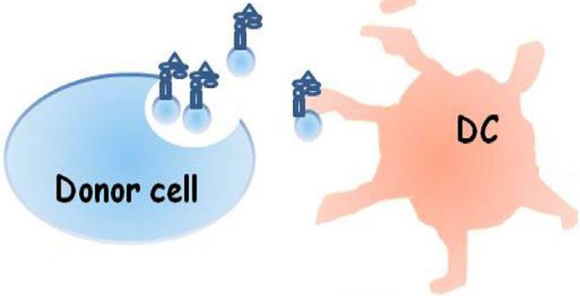


Cross dressing

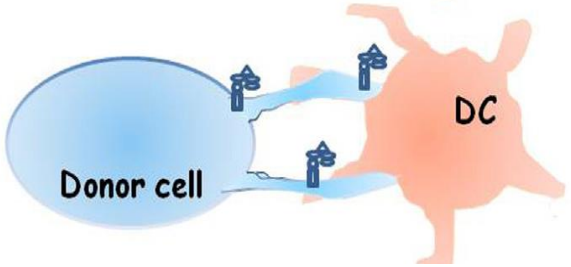
Trogocytosis



Exosomes



Tunneling Nanotubes

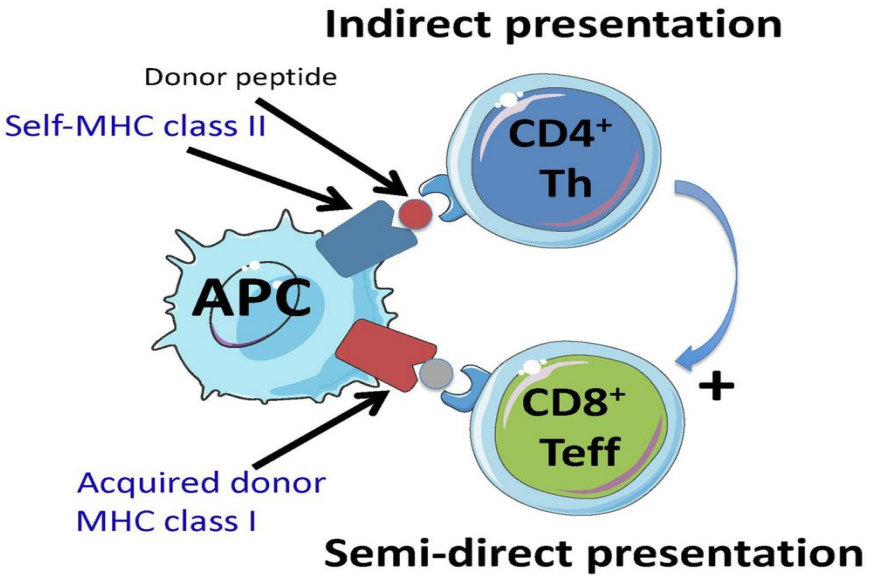


Frelinger Jeffrey-1974

MHC'lerin lökositler arasında transferi

Brian Dolan-2006

Transferden sonra MHC-peptit kompleksleri iki gün boyunca APC de exp.



Lesley Smythe 2013

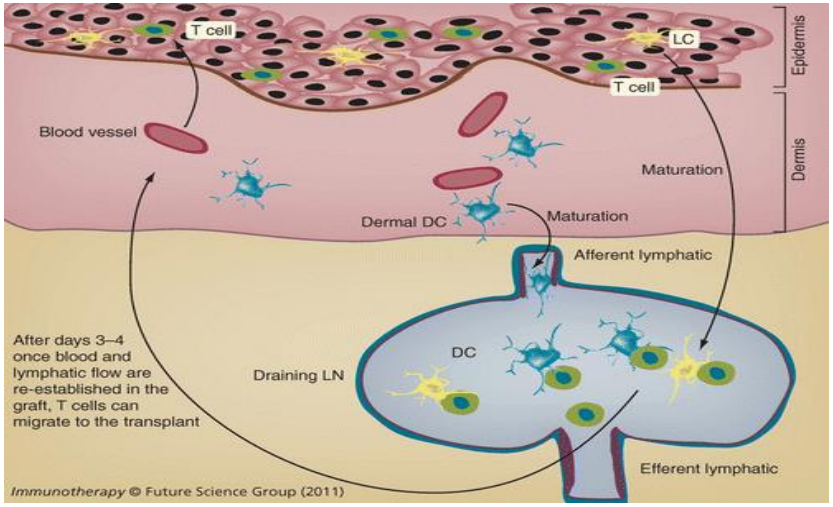
Alıcı DC'leri trf allogeneik MHC-sınıf I kazanımının, transplantasyonu takiben en az 1 ay boyunca gerçekleştiğini ve CD8 T hücre aktivasyonunun temel sebebi olduğunu göstermişlerdir.

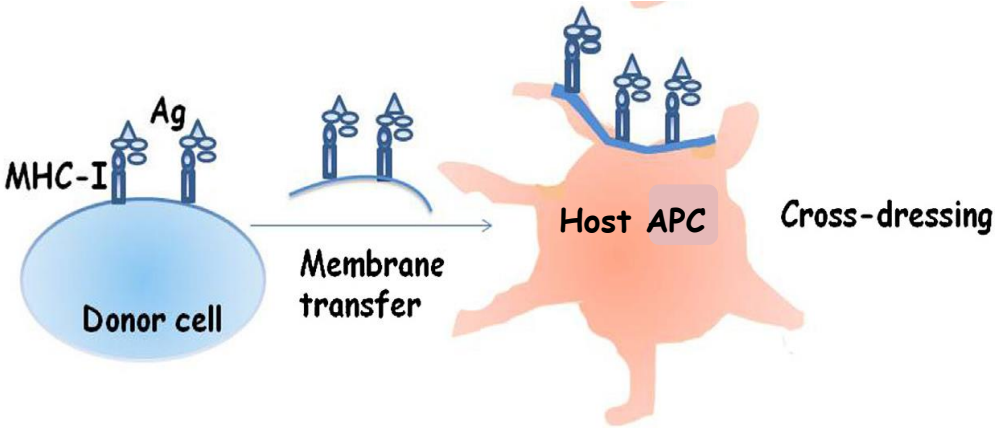
100/1000000 donör DC
90000/1000000 Cross dressing alıcı APC

2016

Jose Marino

Mohamed H. Babiker-Mohamed

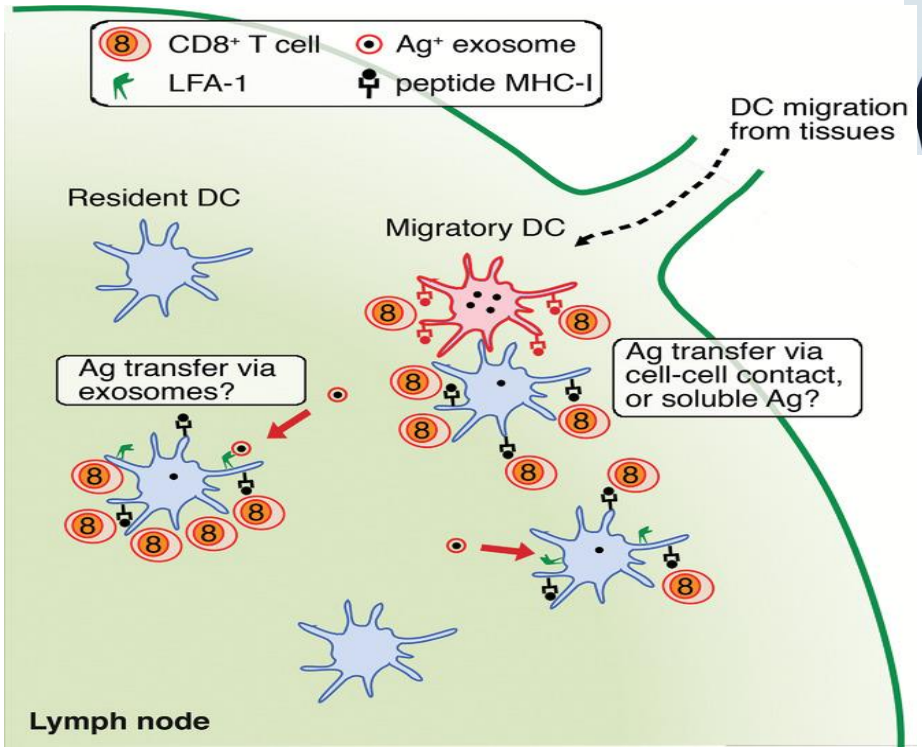




Marino J. Donor exosomes rather than passenger leukocytes initiate alloreactive T cell responses after transplantation, Science Immunology, 2016.

2016

Adrian Morelli

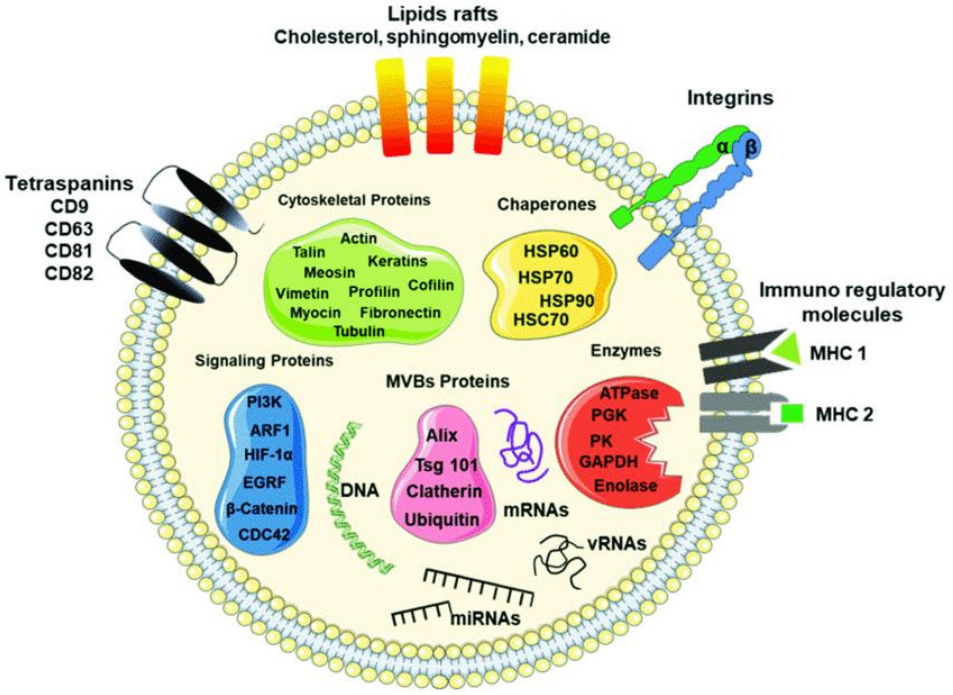


Quan Liu

Liu Q, Morelli AE, Donor dendritic cell-derived exosomes promote allograft-targeting immune response, J Clin Invest, 2016

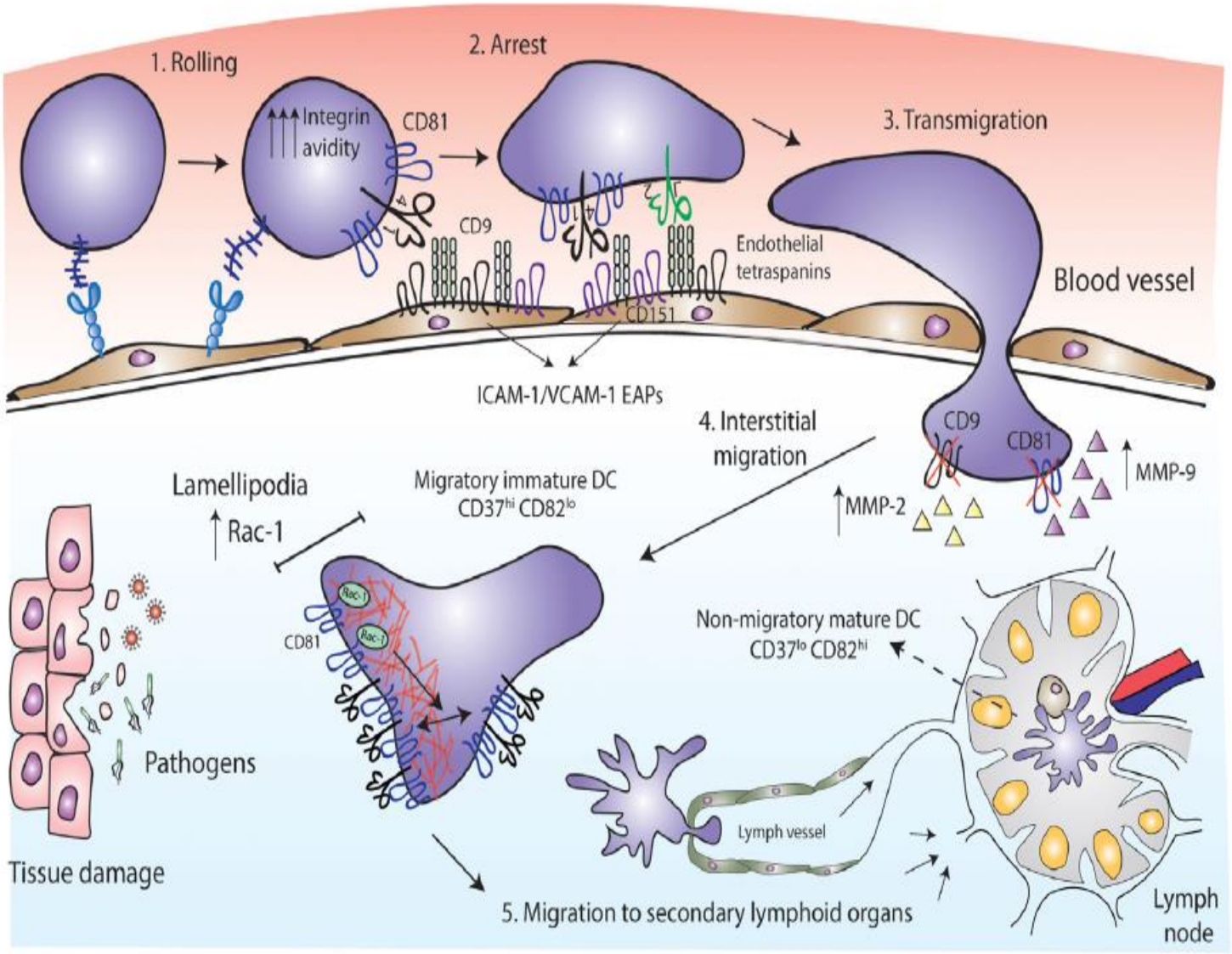
Tetraspaninler

- ✓ PRR
- ✓ MHC II
- ✓ Adezyon molekülleri
- ✓ Sinyal molekülleri

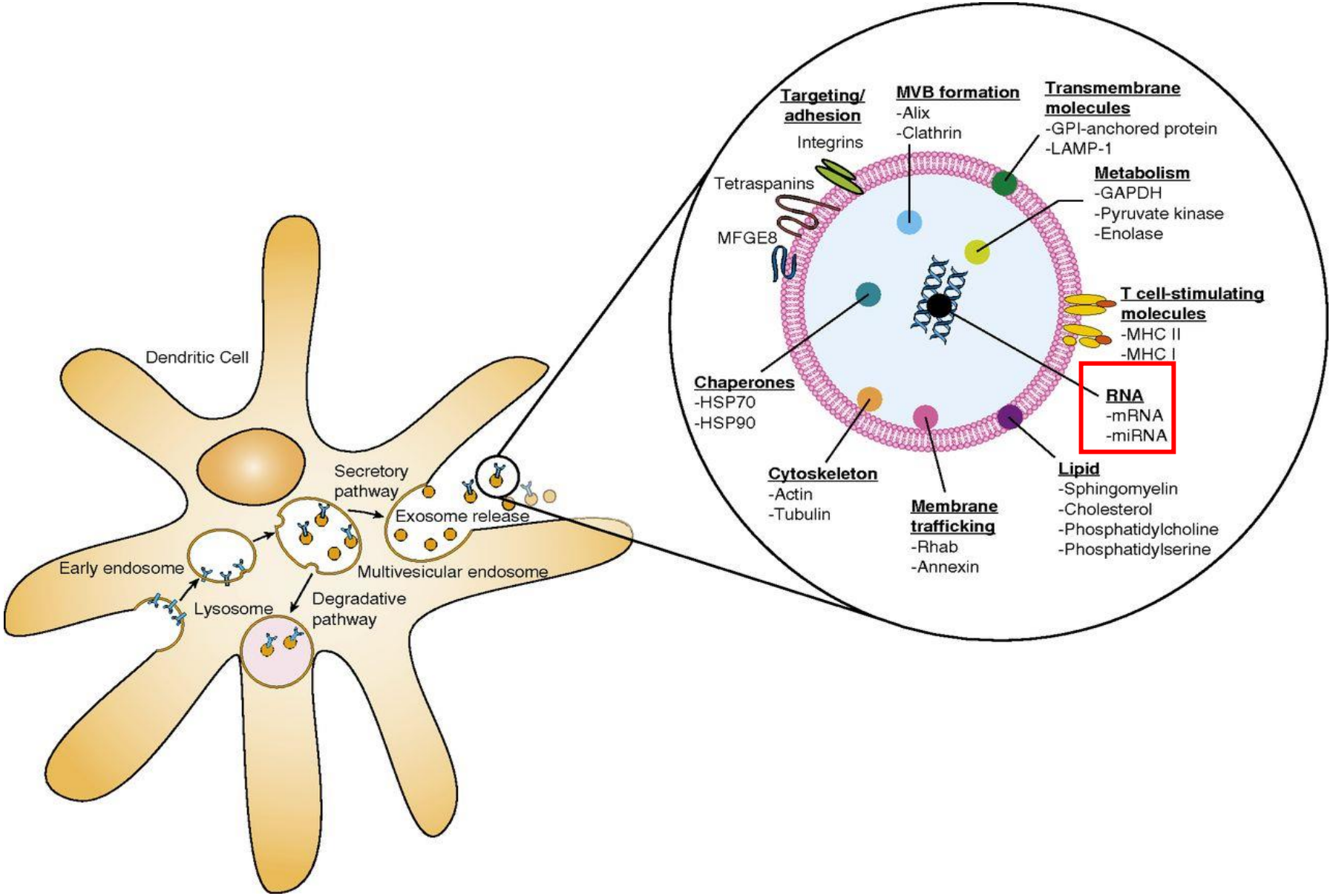


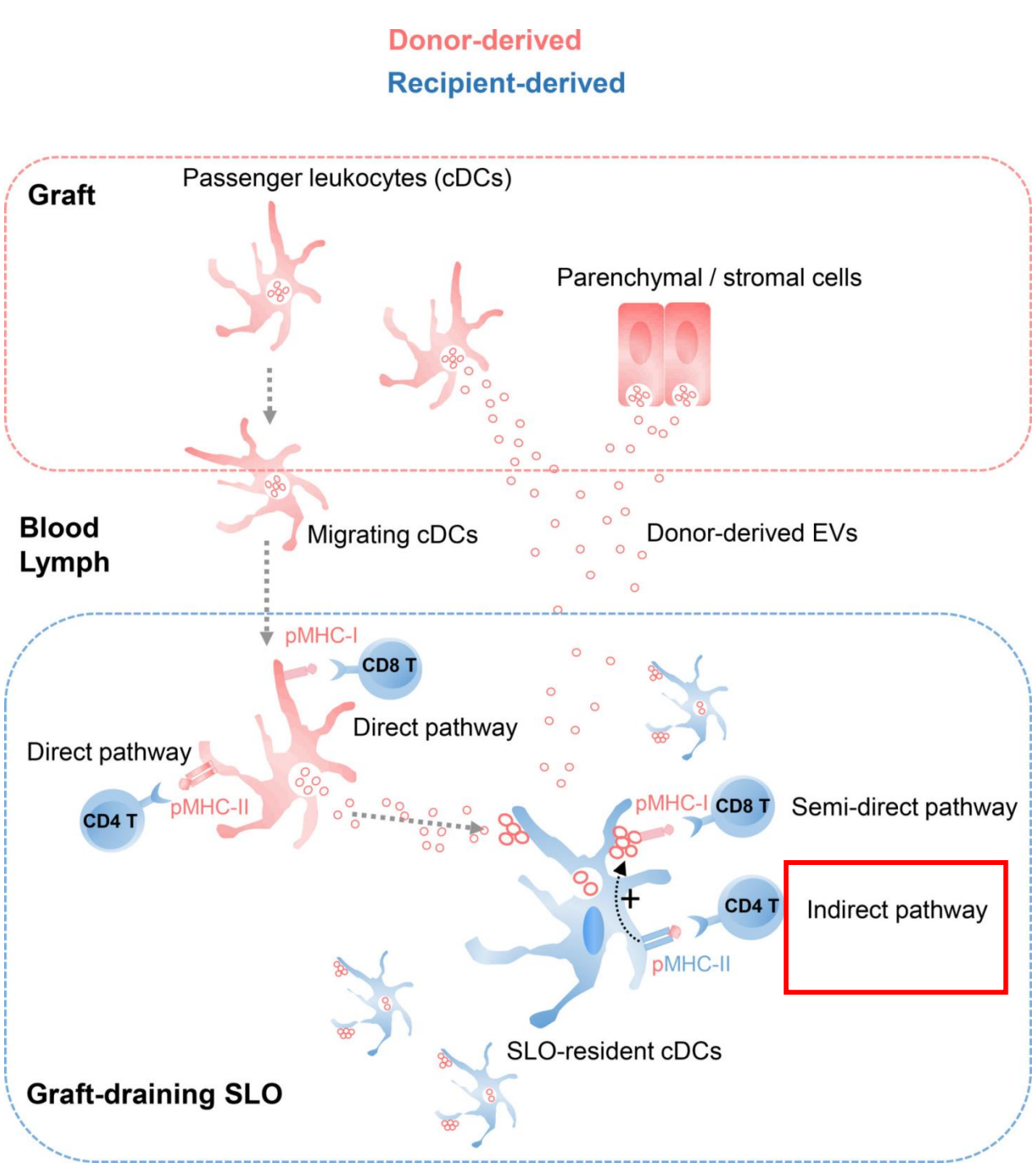
Tetraspanins as Organizers of Antigen-Presenting Cell Function -2018

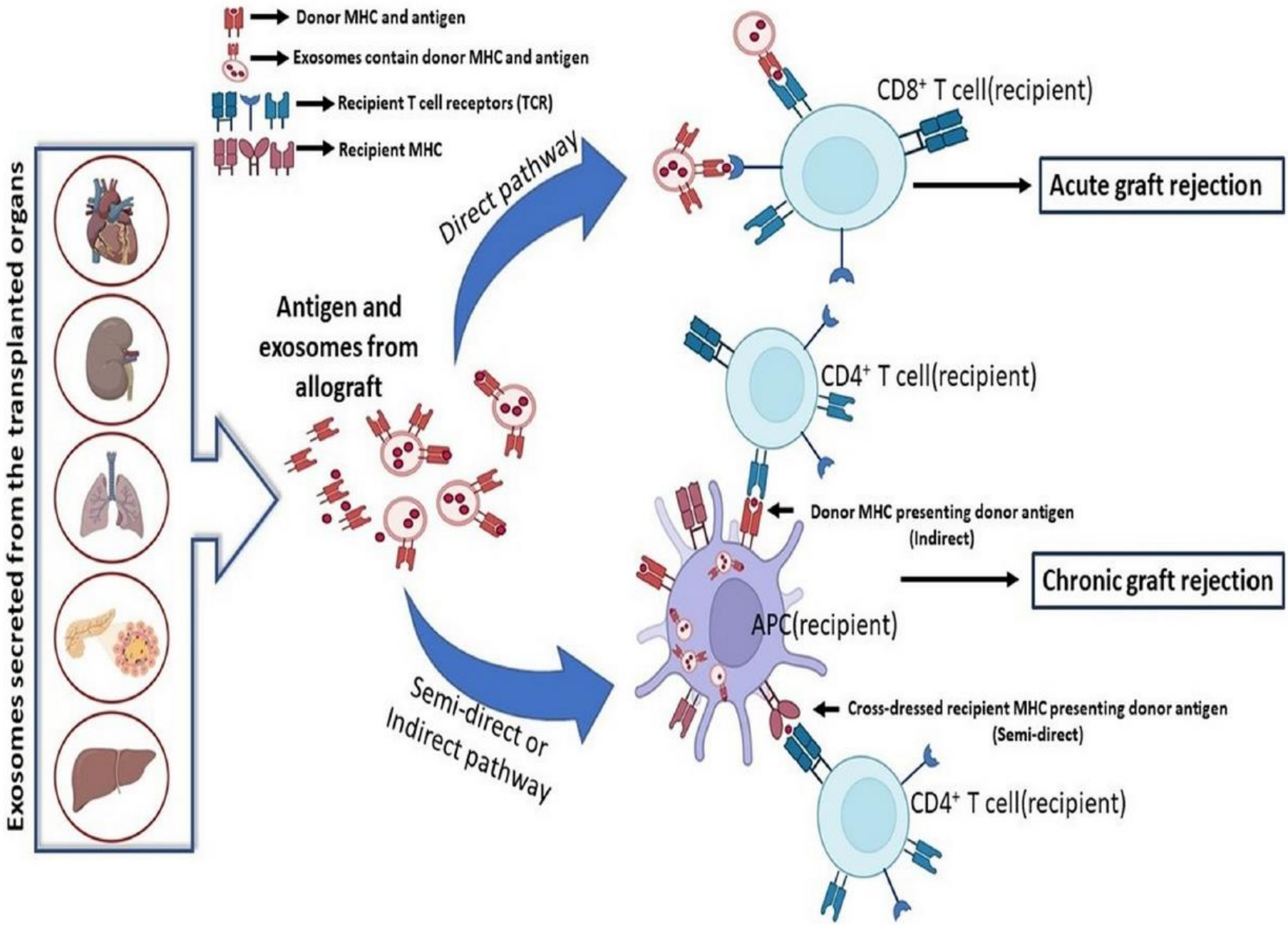
Tetraspanins act as key players in APC migration



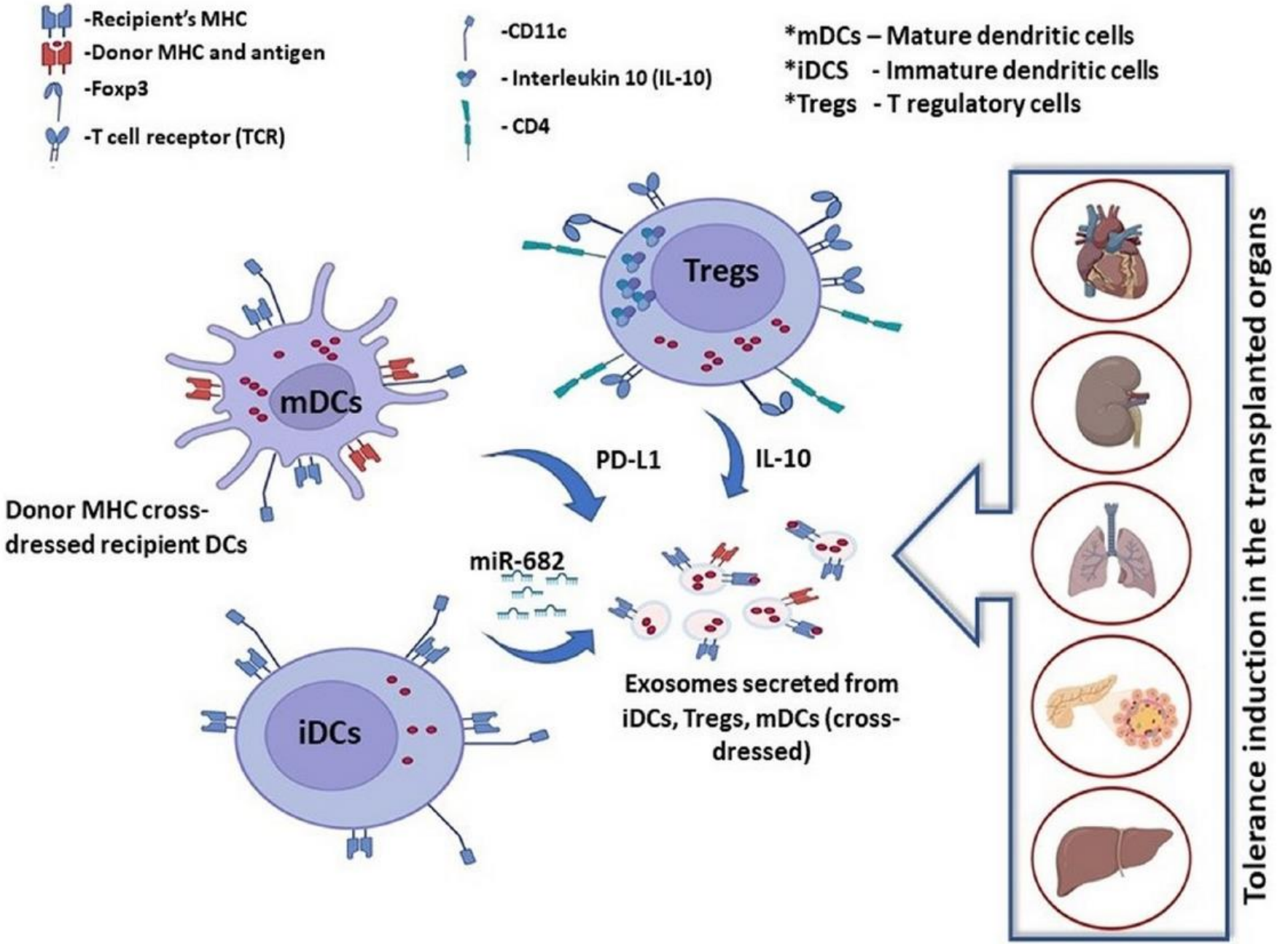
EV'yi salgılayan Hücre!!!!! farklı T hücre yanıtları
EV'lerin transfer ettiği kargo !!!!!!!! Sayı!!!!!!



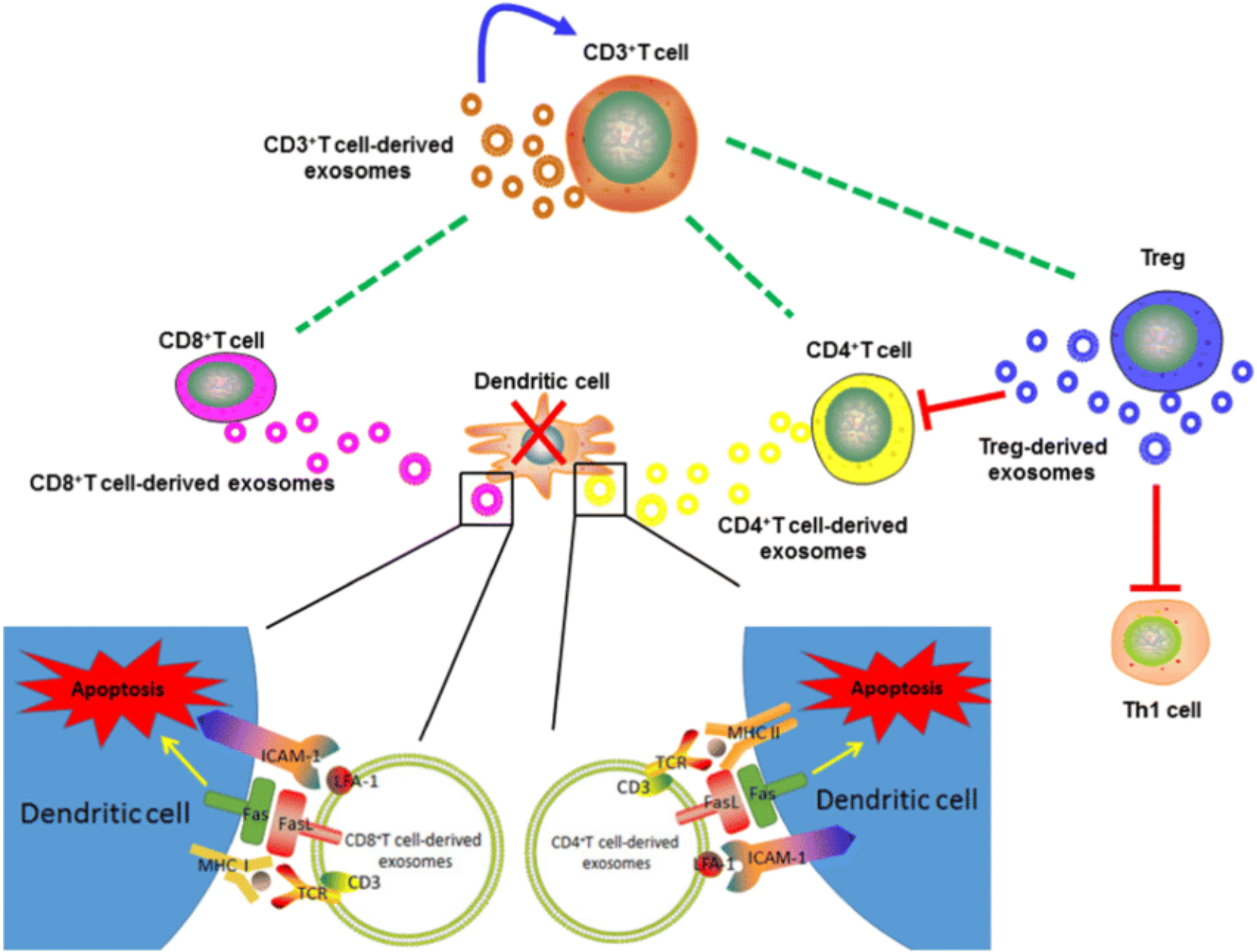

Donor-derived
Recipient-derived
Graft
Passenger leukocytes (cDCs)
Parenchymal / stromal cells
Blood
Lymph
Migrating cDCs
Donor-derived EVs
pMHC-I
CD8 T
Direct pathway
Direct pathway
CD4 T
pMHC-II
pMHC-I
CD8 T
Semi-direct pathway
CD4 T
Indirect pathway
pMHC-II
SLO-resident cDCs
Graft-draining SLO

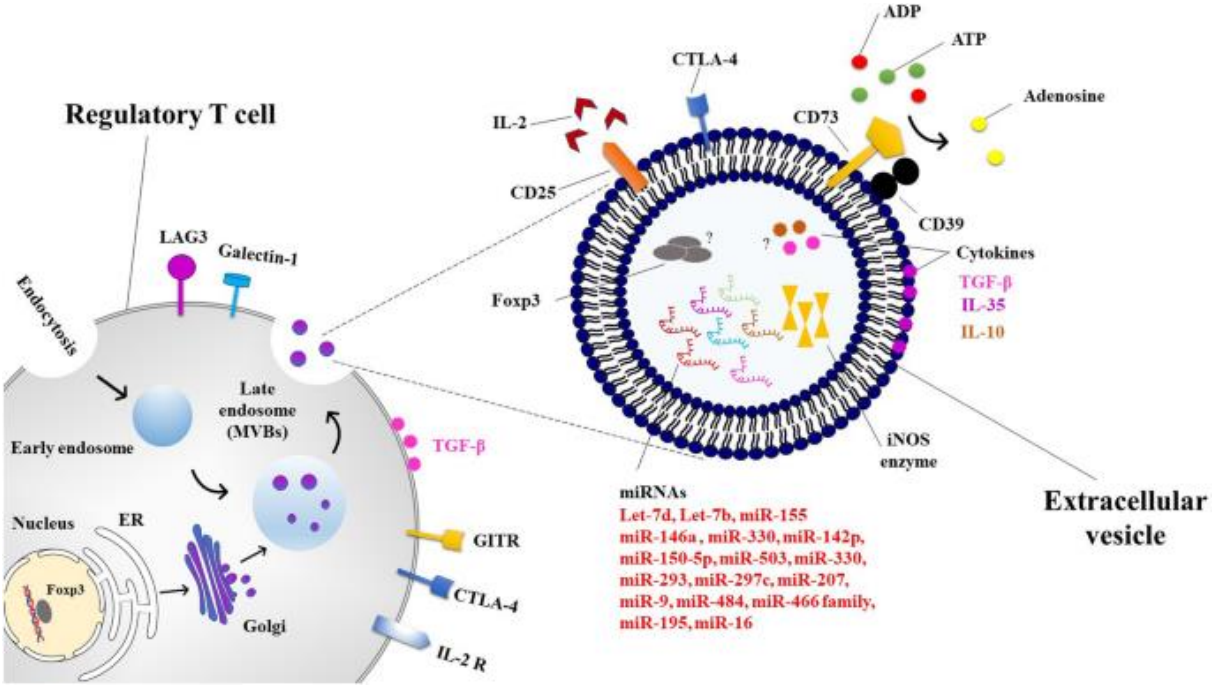


Life Sciences 324 (2023) 121722

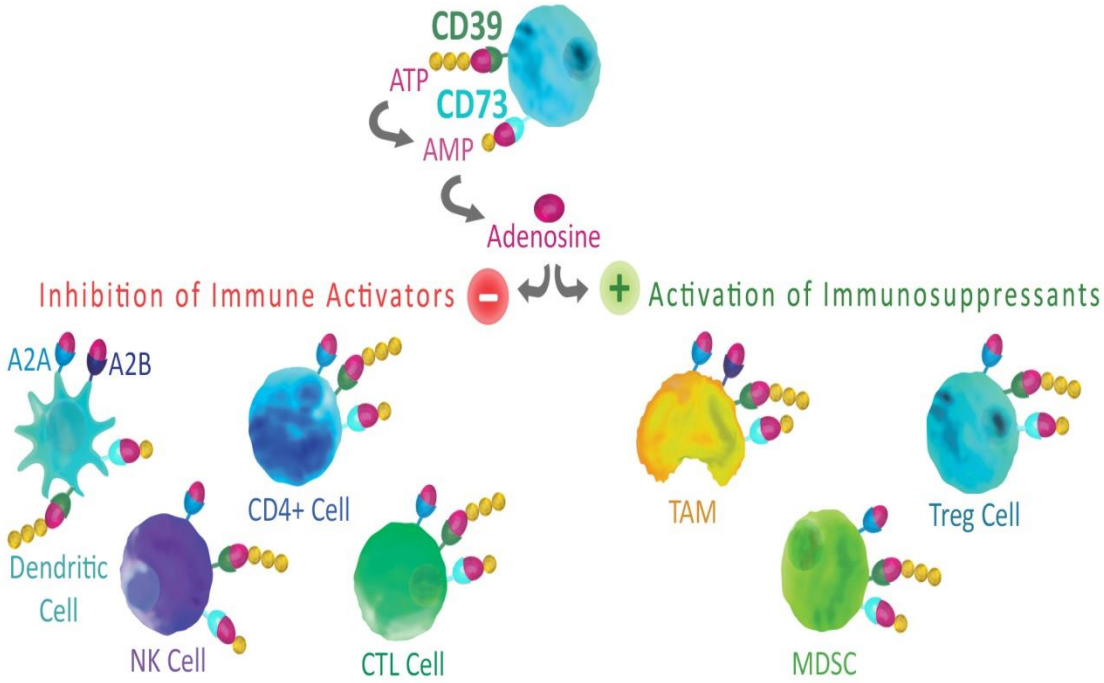


Life Sciences 324 (2023) 121722


CD3⁺T cell
CD3⁺T cell-derived exosomes
Treg
CD8⁺T cell
CD4⁺T cell
Dendritic cell
Treg-derived exosomes
CD8⁺T cell-derived exosomes
CD4⁺T cell-derived exosomes
Th1 cell
Apoptosis
ICAM-1
LFA-1
Fas
FasL
Dendritic cell
CD8⁺T cell-derived exosomes
MHC I
TCR
CD3
MHC II
TCR
CD3
FasL
Fas
Apoptosis
Dendritic cell
CD4⁺T cell-derived exosomes
LFA-1
ICAM-1



Kompleman pro
Kostimulatörler



Non invasif tanı aracı olarak Exosomlar

Kan,

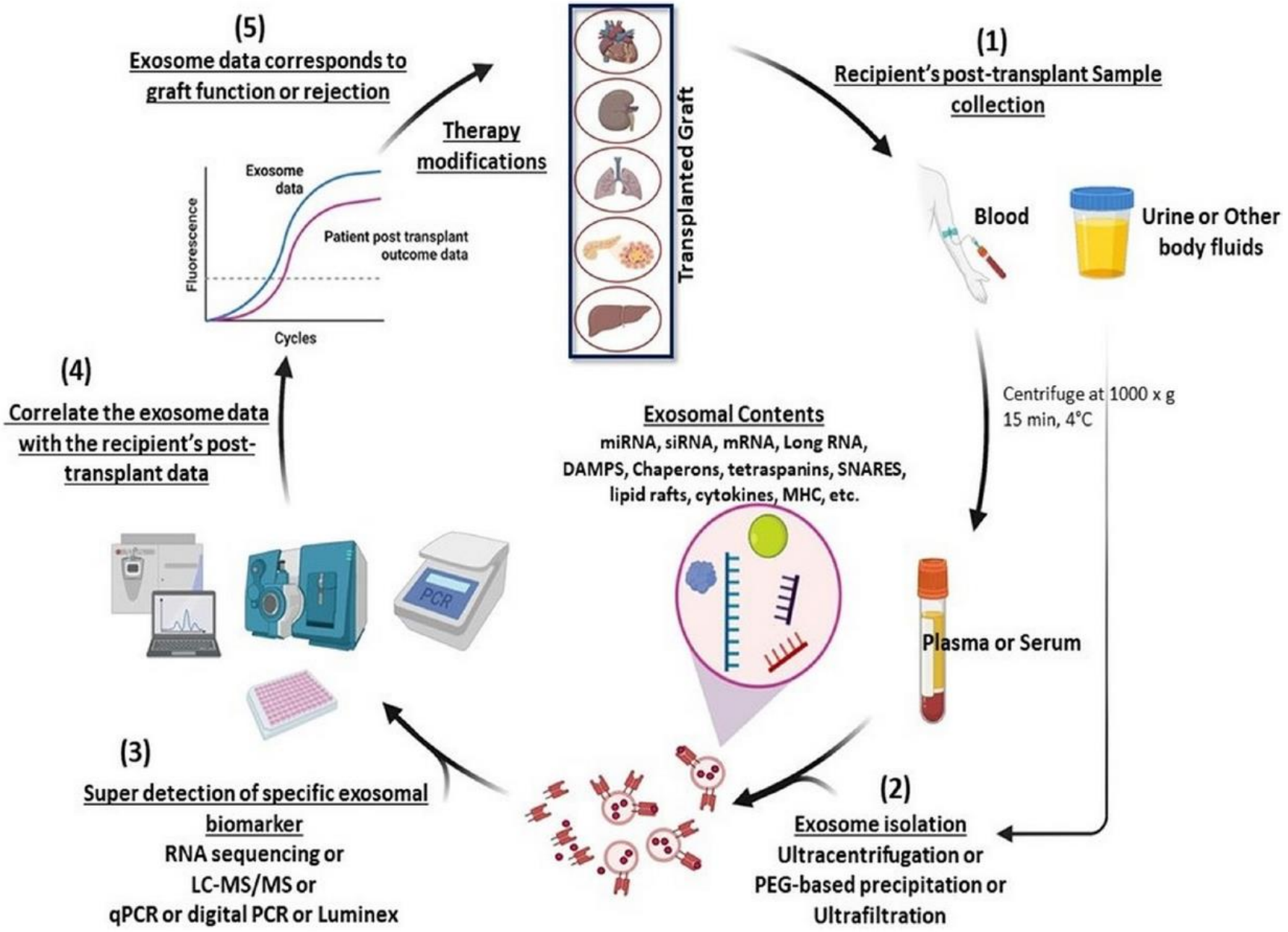
İdrar

Bronkoalveolar lavaj (BAL),

Tükürük,

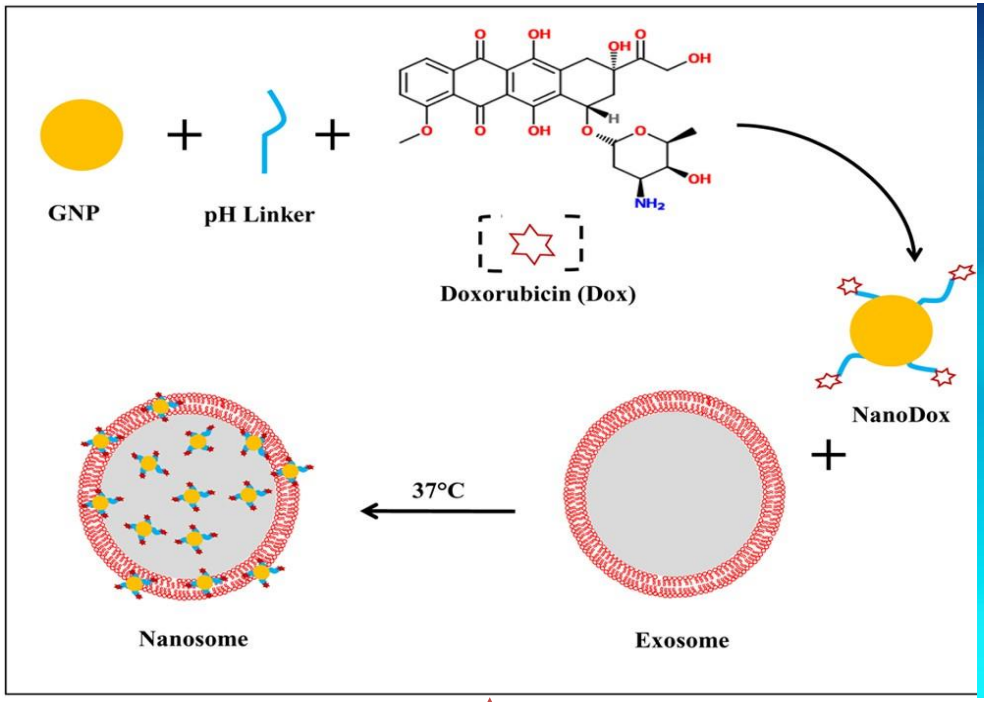
Süt,

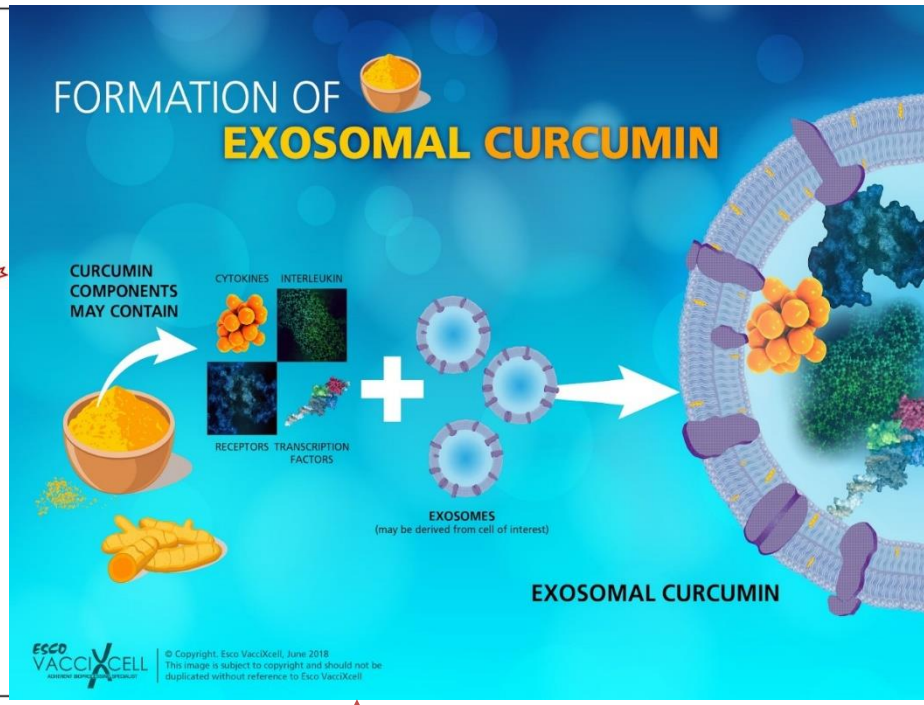
Amniyotik sıvı


(5)
Exosome data corresponds to graft function or rejection
Therapy modifications
Exosome data
Patient post transplant outcome data
Fluorescence
Cycles
Transplanted Graft
(1)
Recipient's post-transplant Sample collection
Blood
Urine or Other body fluids
Centrifuge at 1000 x g
15 min, 4°C
Exosomal Contents
miRNA, siRNA, mRNA, Long RNA,
DAMPS, Chaperons, tetraspanins, SNARES,
lipid rafts, cytokines, MHC, etc.
Plasma or Serum
(4)
Correlate the exosome data with the recipient's post-transplant data
PCR
(3)
Super detection of specific exosomal biomarker
RNA sequencing or
LC-MS/MS or
qPCR or digital PCR or Luminex
(2)
Exosome isolation
Ultracentrifugation or
PEG-based precipitation or
Ultrafiltration

Exosome temelli tedaviler

- Lipit bariyer ilacın korunması açısından önemli
- Alıcı hücrelere endositozu kolaylaşır.
- Exosomların düşük immunojenitesi yan etkiyi azaltır.
- Kan beyin bariyerini geçer





Anti kanser etki ↑

Yan etki ↓

Stabilite ↑

Kan konsantrasyonunda ↑

Lipopolisakkaritlerle indüklenen septik şoka karşı koruyucu

Table 1
Recipient's exosomes were used as a potential biomarker in clinical transplantation.

	Transplant Type	Methods used	The outcome of the study
1	Kidney	LC-MS/MS	➢ The global protein contents were profiled from the exosomes isolated from the urine samples of transplant recipients ➢ About 17 proteins were seen to increase in the exosomes of the transplant recipients diagnosed with T cell-mediated rejection (TCMR) ➢ Tetraspanin-1 and hemopexin in the exosomes were significantly higher in TMCR recipients [33].
2	Kidney	qPCR	➢ Total RNA was extracted from the plasma samples collected from the transplant recipients ➢ About 21 candidate mRNA levels are high in the exosomes of recipients diagnosed with antibody-mediated rejection (AMR) ➢ Among them, gp130, SH2D1B, TNFα, and CCL4 mRNAs were significantly higher in AMR cohort exosomes than in other groups [32].
3	Kidney	iKEA (Biochip)	➢ Exosomes were directly captured from the urine samples collected from the recipients ➢ The ikea captured high levels of CD3 (T- cell marker) positive exosomes in allograft-rejecting recipient's urine samples with 91.1 % accuracy. [72]
4	Kidney	qPCR (TaqMan)	➢ Exosomes were isolated from the recipient urine samples and the miRNAs were quantified using TaqMan qPCR ➢ The elevated amount of BK viral miRNAs B1-5p and 3p in the urine exosomes of renal transplant recipients with BK virus nephropathy (BKVN). ➢ These exosomal viral miRNAs could be used as a surrogate marker for the diagnosis of BKVN [116]

Table 1 (*continued*)

	Transplant Type	Methods used	The outcome of the study
5	Pancreatic Islet	HLA-specific NTA and Immunoblotting	➢ Quantitation and characterization of allo-islet exosomes in the recipient's plasma ➢ The donor islet-specific exosome numbers decreased in the recipient plasma before islet graft dysfunction ➢ The donor-specific islet exosomes contain the exosome markers (CD63, flotillin), and transmembrane protein (FXYD2) along with donor-specific HLA molecules. [71]
6	Pancreatic Islet	NTA	➢ Quantitation and characterization of allo-islet exosomes in the recipient's plasma ➢ There is time specific increase of auto antigen GAD65 in the plasma exosomes of these recipients indicating the recurrence of autoimmunity in T1D recipients after islet transplantation [117]

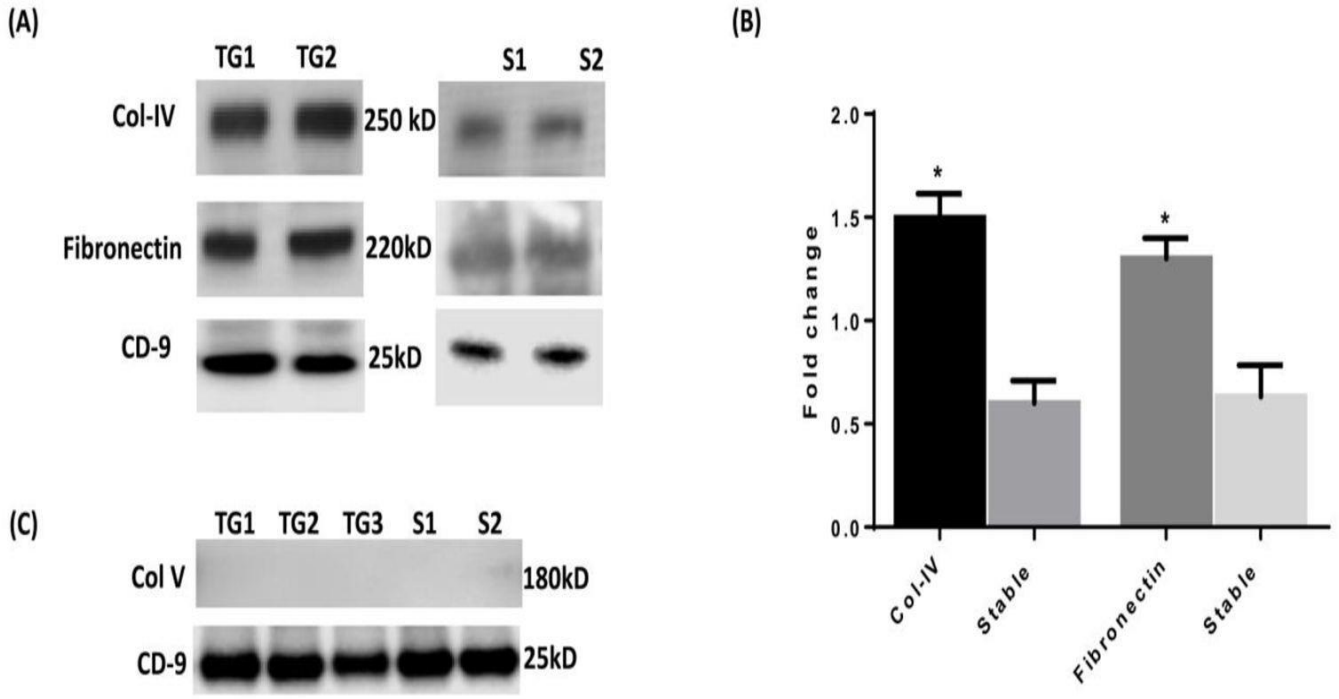
(*continued on next page*)

Table 1 (*continued*)

	Transplant Type	Methods used	The outcome of the study
7	Pancreatic Islet	RNA sequencing and qPCR	➢ Total RNAs were isolated from the Islet transplant recipient's plasma and miRNAs present were profiled using RNA sequencing and qPCR ➢ Islets release a significant amount of ER stress-induced miRNAs (miR-29b and miR-216a) and damage associated miRNAs (miR-375 and miR-148a) to the circulation during peritransplant time through exosomes ➢ The high amount of these exosomes release during the peritransplant period correlated with poor transplant outcomes in Islet transplants. [7,118]
8	Pancreatic Islet	LC-MS/MS and ELISA	➢ Proteomic profile of the exosomes isolated in samples collected during clinical islet isolations ➢ Analysis of the global protein content in the exosomes isolated during isolation reveals a high amount of DAMPs like histone (H2B, H3, and H4) and Chaperons (HSP 70, HSPA1) ➢ The high amount of DAMPs/Islet equivalence (IEQ) released during isolation positively correlated with the recipient's post-transplant insulin requirement [80,81]
9	Heart	LC-MS/MS	➢ Proteomic profiling of serum exosomes of the allograft-rejecting recipients. Further comparative pathway analysis revealed the possible mechanisms regulated by these miRNAs such as Pi3K-AKT, Wnt, endocytosis, focal adhesion, ubiquitin-mediated proteolysis, MAPK kinase, and TGF-β. [18]
12	Lung	Luminex, ELISPOT, and Immunoblotting	➢ The exosomes were isolated from the transplant recipient's serum and BALF ➢ The exosomes isolated from the serum of lung transplant recipients with BOS showed a significantly high amount of SAgs, Col-V, Kα1T, 20s proteasome α3 subunit, along with costimulatory molecules (CD80, CD86, CD40, MHC-II), and transcription factors (NF-kB, HIF-1A, MHC CIITA, IRAK1, and MyD88) [17,119,120]
13	Lung	RNA sequencing	➢ The exosomes were isolated from the transplant recipient's BALF. The Global RNA content was profiled. ➢ About 29 inflammatory and immune-related RNAs were seen significantly high in the exosomes isolated from the BALF of recipients with acute rejection. ➢ These exosomal RNAs could be a possible biomarker for acute rejection [121]

Table 1 (*continued*)

	Transplant Type	Methods used	The outcome of the study
9	Heart	LC-MS/MS	➢ Proteomic profiling of serum exosomes isolated from transplant recipients ➢ About 15 proteins were differentially expressed in the exosomes of the allograft rejected recipients compared to non-rejected recipients. ➢ Within these 15 proteins, 8 proteins were known for their role in immune regulation, complement activation, adaptive immunity, and coagulation. [75]
10	Heart	qPCR array	➢ Serum exosomes were isolated from the transplant recipients and the miRNA present were profiled using qPCR array ➢ The exosomes isolated from the allograft acute rejecting recipients showed enrichment of 4 miRNAs (miR-142, miR-92a, miR-339, and miR-21). ➢ The study has identified a novel mechanism of miR-142, which is transferred to endothelial cells from acute rejecting allograft exosomes. MiR-142 regulates the recipient endothelial function by targeting RAB11FIP2. [27]
11	Lung	qPCR array and immune blotting	➢ The exosomes were isolated from the transplant recipient's serum and bronchoalveolar lavage fluid (BALF) ➢ Donor HLA and lung-associated self-antigens (SAgs), Collagen V (Col-V), and Kα1 tubulin were detected in the exosomes of allograft-rejecting recipients only. ➢ About 123 miRNAs were differentially expressed in the



Tissue-associated self-antigens containing exosomes: Role in allograft rejection-2018

Teşekkürler