质谱技术在EVs检测中的应用

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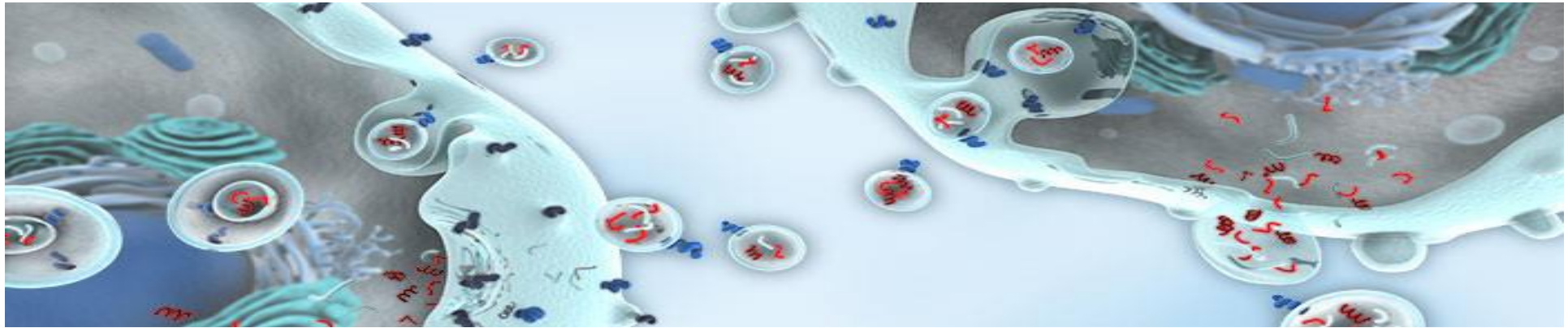
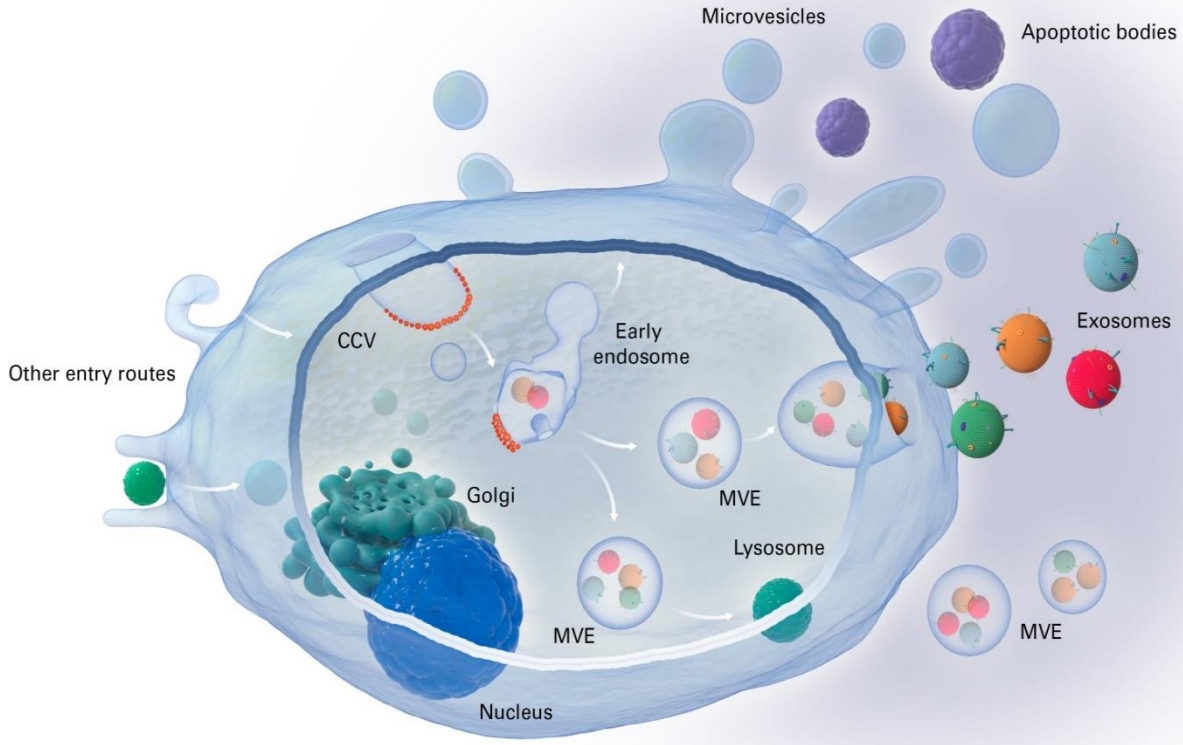


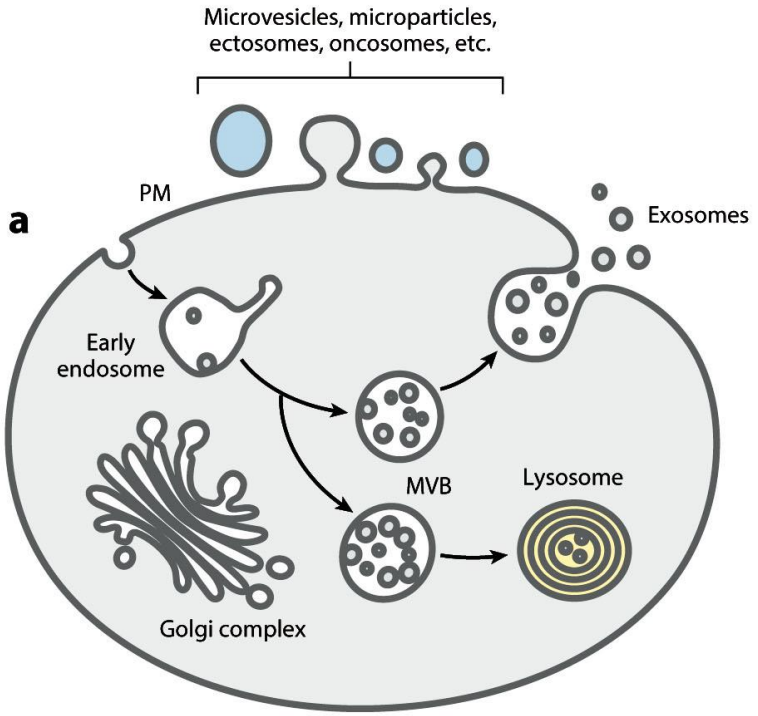
目录

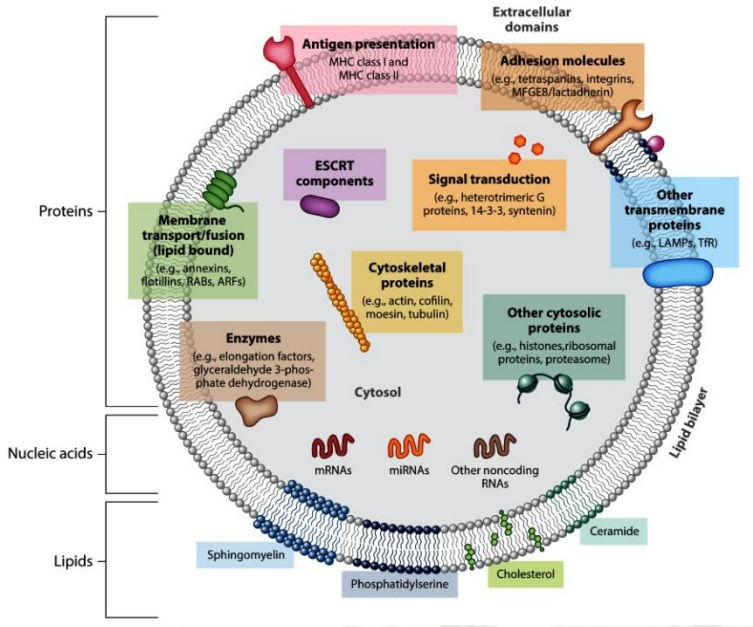
1. 细胞外囊泡（EVs）简介

所有细胞都分泌细胞外囊泡（Extracellular Vesicles）



细胞外囊泡（Extracellular Vesicles）

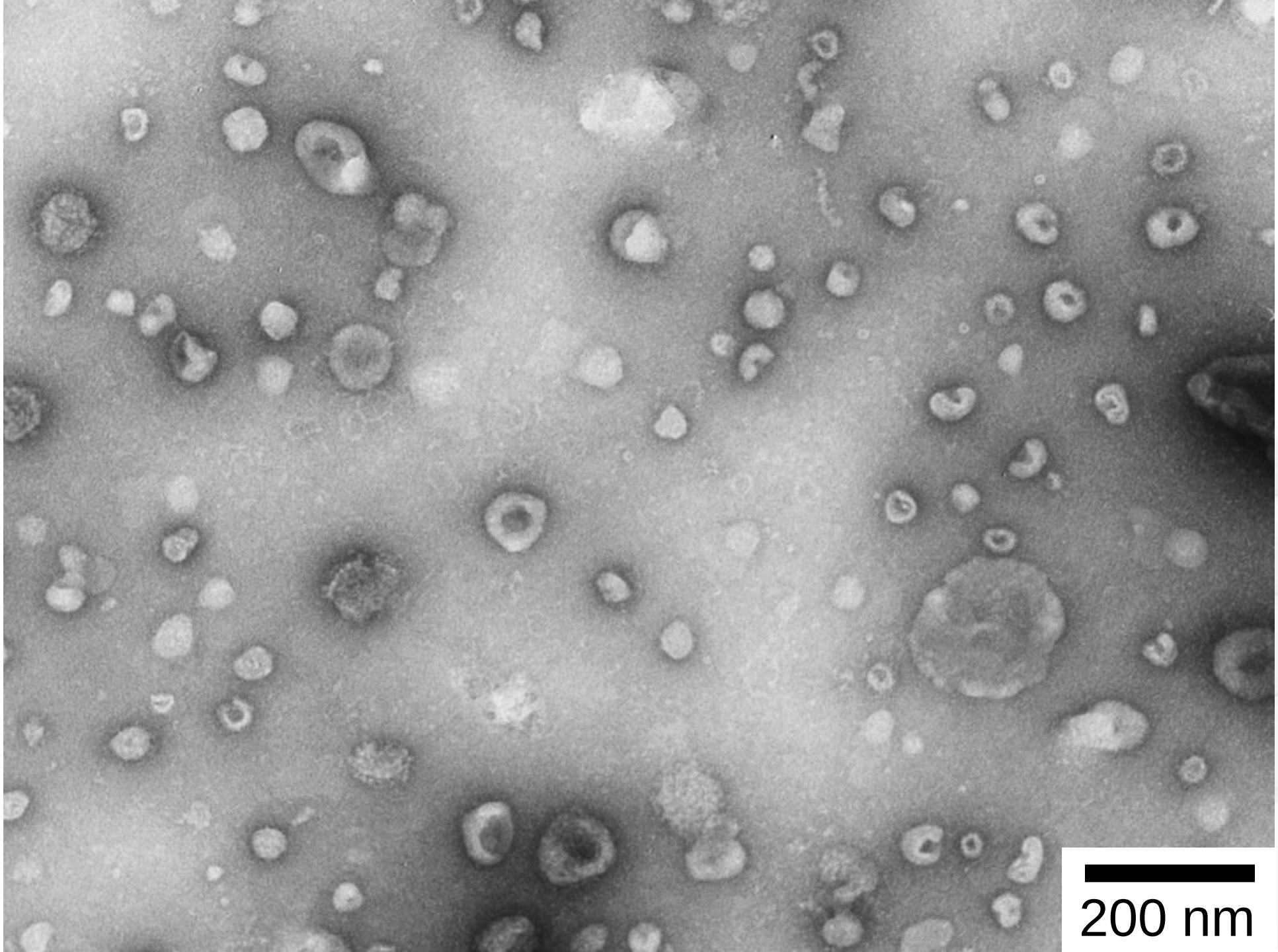




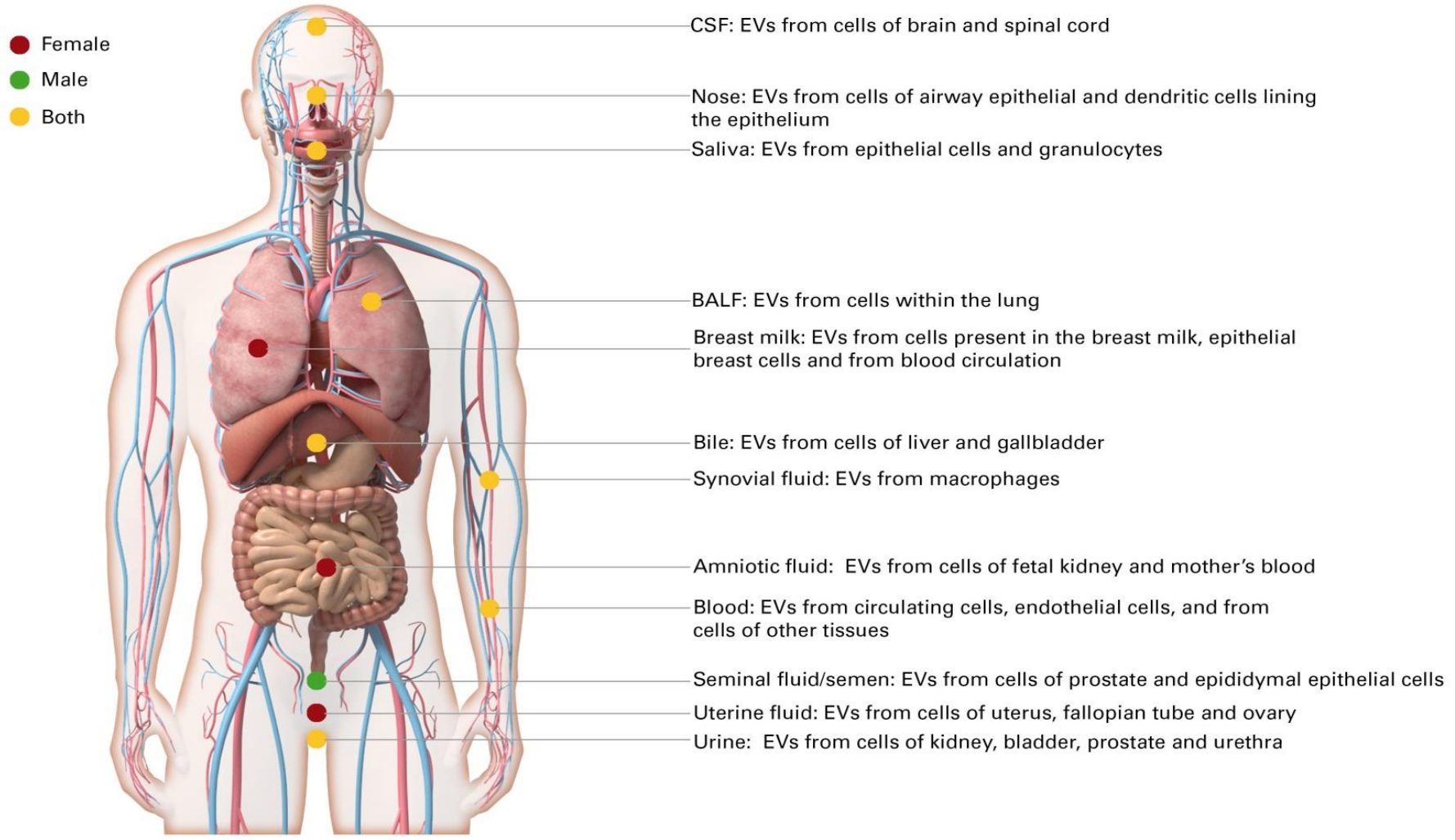
细胞外囊泡（EVs)是细胞在向外出芽和细胞膜裂变的过程中释放出30-2000nm的微小囊泡；

EVs内含多种生物学活性物质如：核酸、蛋白质、脂类等

Marina Colombo et al, Annual Review Cell Developing Biology (2014)

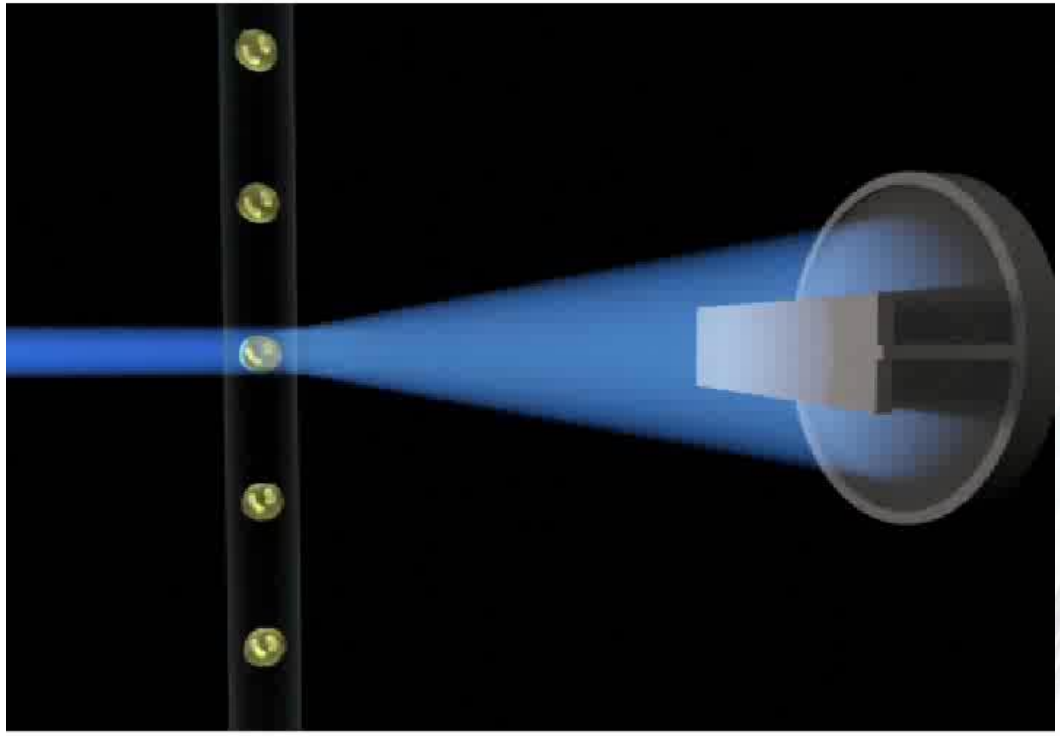

200 nm

EV 的体内分布


Female
Male
Both
CSF: EVs from cells of brain and spinal cord
Nose: EVs from cells of airway epithelial and dendritic cells lining the epithelium
Saliva: EVs from epithelial cells and granulocytes
BALF: EVs from cells within the lung
Breast milk: EVs from cells present in the breast milk, epithelial breast cells and from blood circulation
Bile: EVs from cells of liver and gallbladder
Synovial fluid: EVs from macrophages
Amniotic fluid: EVs from cells of fetal kidney and mother's blood
Blood: EVs from circulating cells, endothelial cells, and from cells of other tissues
Seminal fluid/semen: EVs from cells of prostate and epididymal epithelial cells
Uterine fluid: EVs from cells of uterus, fallopian tube and ovary
Urine: EVs from cells of kidney, bladder, prostate and urethra

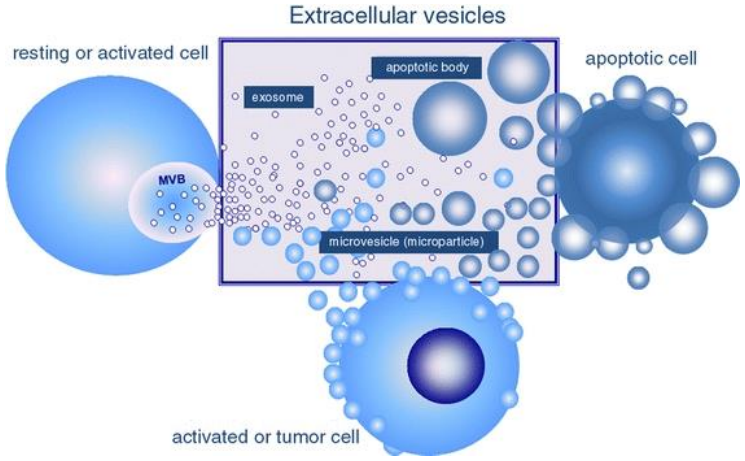
我们能看见EVs吗?

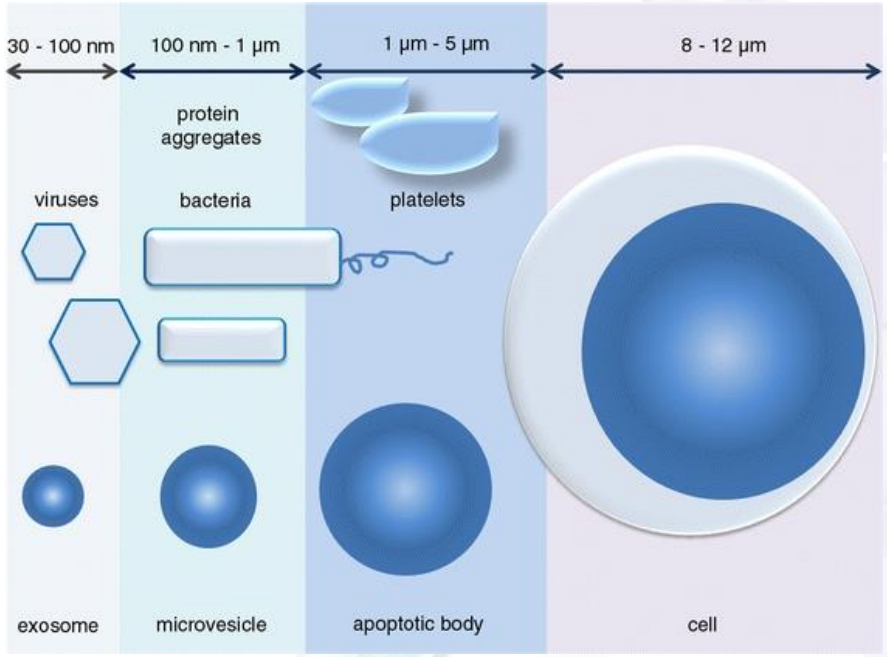
我们能看见EVs吗?



EVs的种类

- 外泌体（Exosome）
- 微泡（Microvesicles）
- 凋亡小体（Apoptotic bodies）





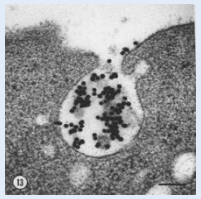
Bence Gyorgy et al, Cellular and Molecular Life Sciences (2015)

观察到EVs

“the clotting factor of which the plasma is deprived”

1946
Chargaff and West

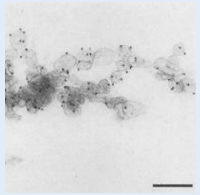
细胞释放



convincingly demonstrate vesicle secretion by cells

1983
Harding et al.

抗原提呈



Exosomes may play a role in antigen presentation

1996
Raposo et al.

ISEV成立

International Society for Extracellular Vesicles were founded

2011
ISEV

第一款诊断试剂

exosomedx ExoDx Lung(ALK)

World’s First Exosomal RNA-Based Liquid Biopsy

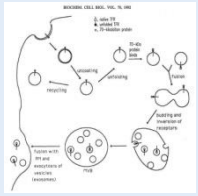
2016
ExoDx, Inc

1967
Wolf

20-50nm, 1.020-1.025 g/ml, “platelet dust”

物理性质

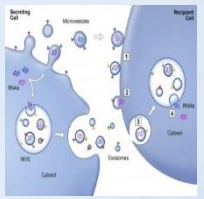
1992
Johnstone et al.



MVB associated with Exosome formation and secretion

释放机制

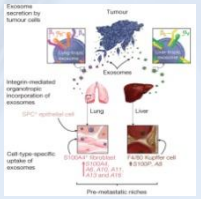
2006
Lotvall et al.



Exosomes transfer mRNA and miRNA between cells

水平转运

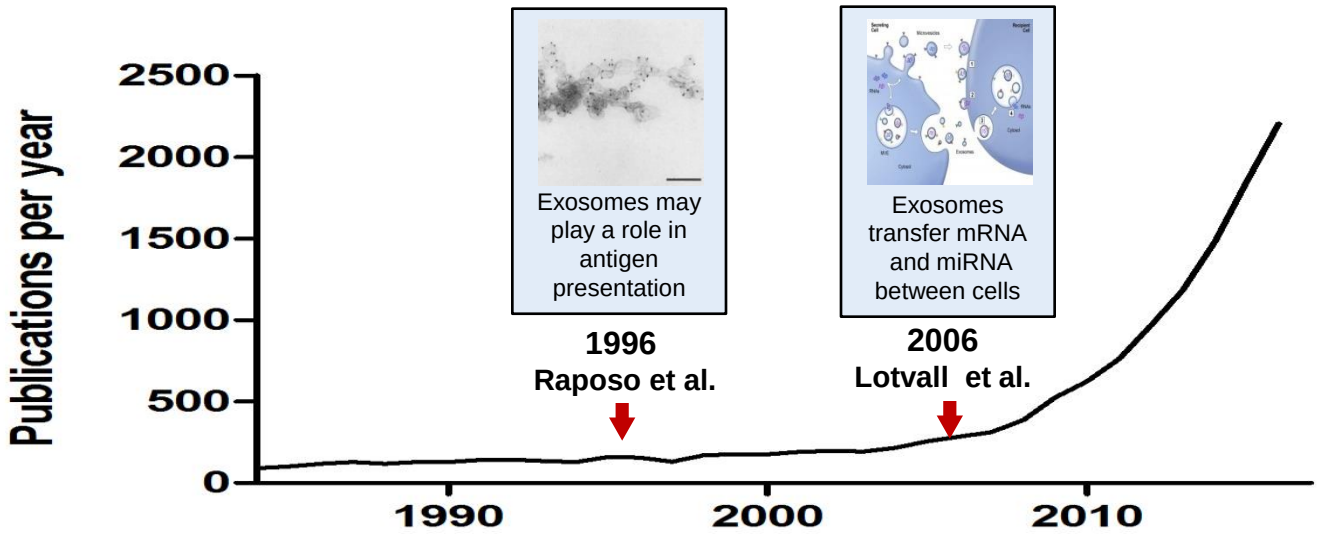
2015
Lyden et al.

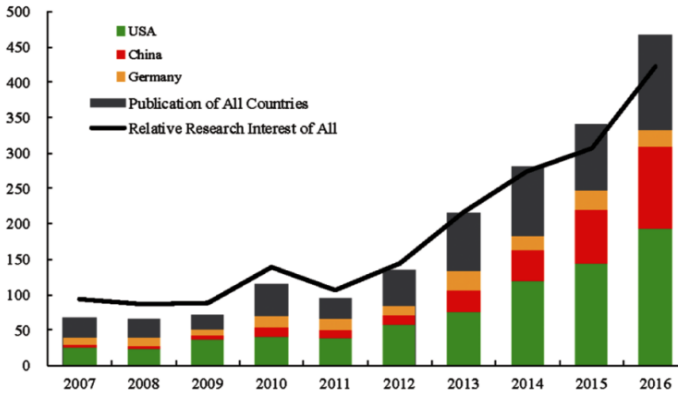


Tumour exosome integrins determine organotropic metastasis

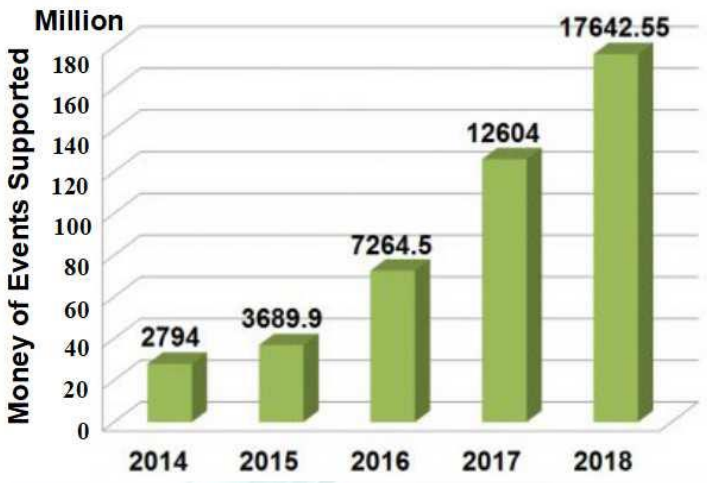
肿瘤转移

EVs的研究热潮



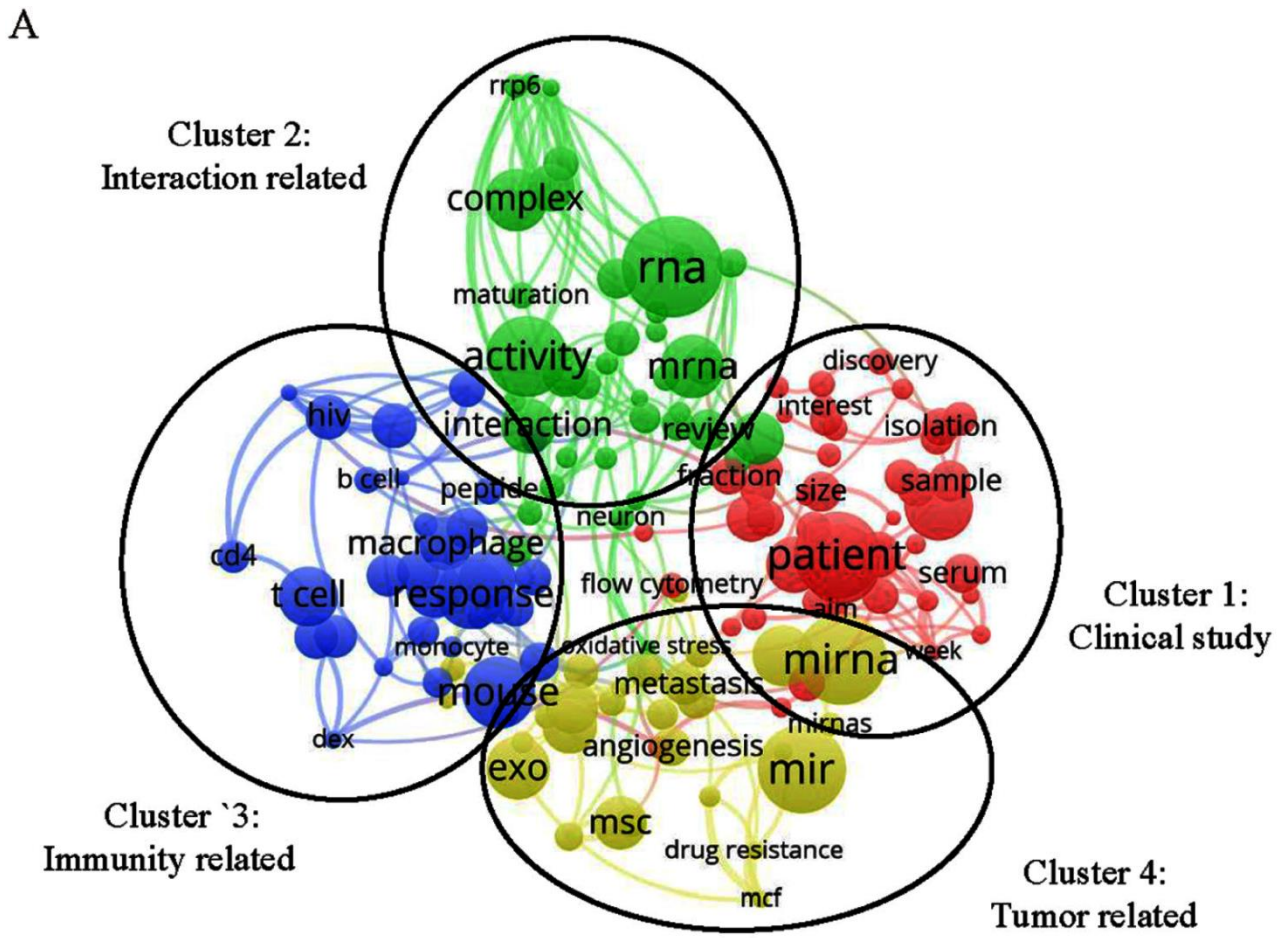


EV相关文章



NSFC 资助

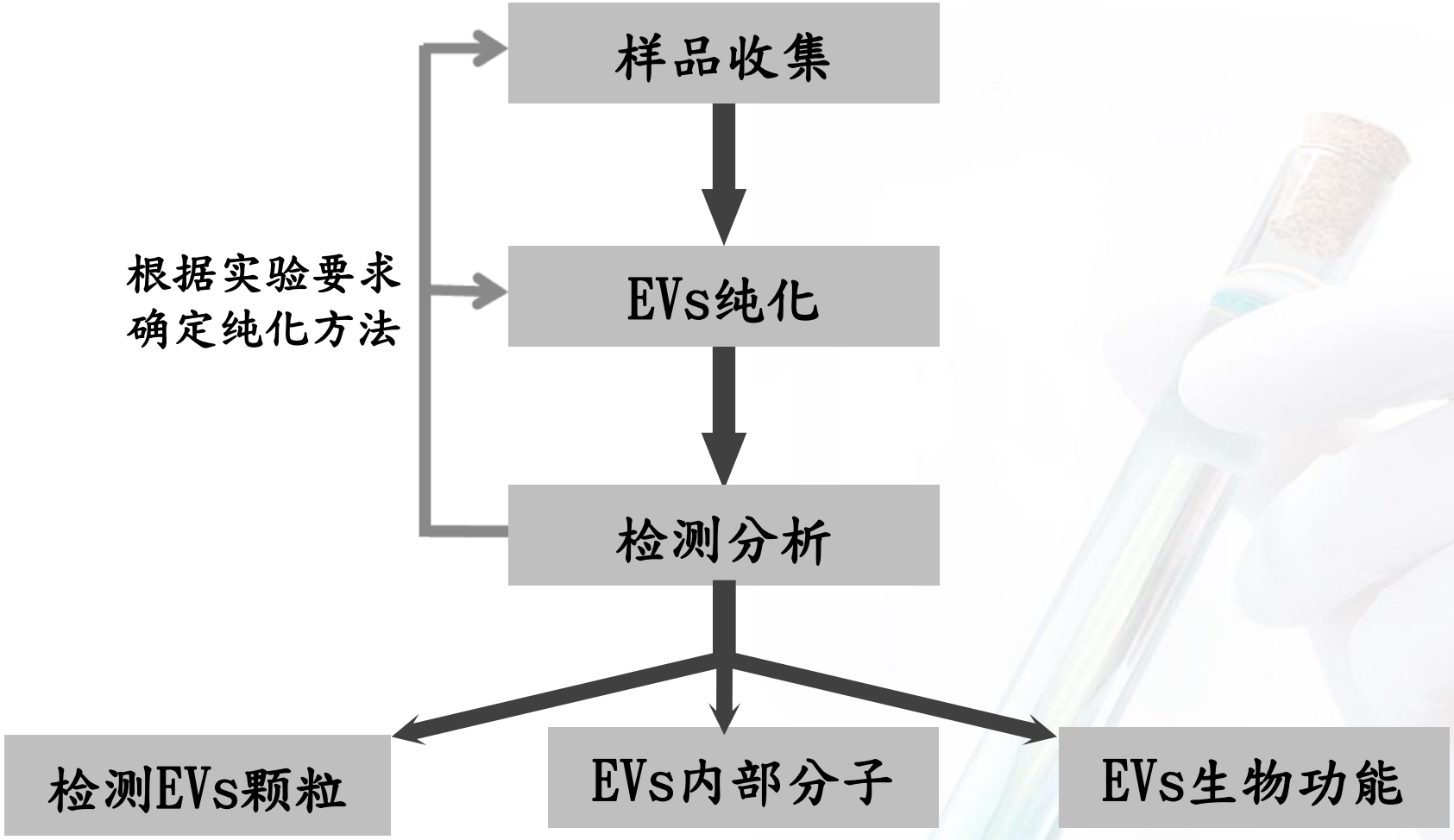
EVs的研究方向



研究较多的方向：临床应用，肿瘤，免疫，细胞间相互作用

Wang Yiran et al, Oncotarget (2017)

EVs的常见研究策略



EVs 研究指南： ISEV, ISTH, AHA

Standardization of sample collection, isolation and analysis methods in extracellular vesicle research.
J Extracell Vesicles, 2013.

Standardization of pre-analytical variables in plasma microparticle determination: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop.
J Thromb Haemost, 2013.

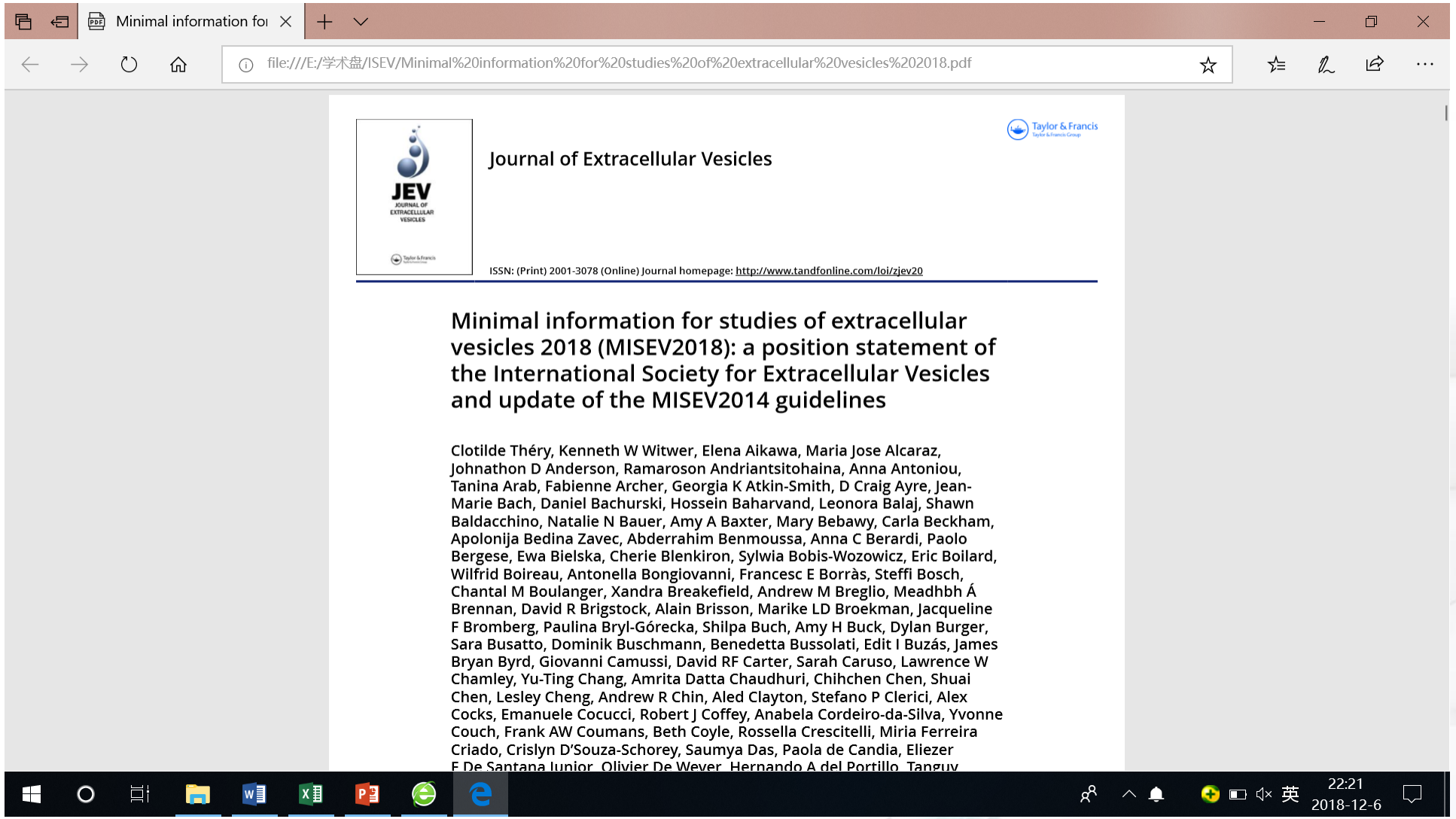
Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles.
J Extracell Vesicles, 2014. 2017. 2018

Obstacles and opportunities in the functional analysis of extracellular vesicle RNA - an ISEV position paper. J Extracell Vesicles, 2017.

EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research.
Nat Methods, 2017.

Methodological Guidelines to Study Extracellular Vesicles. Circ Res, 2017.

MISEV2018 *JEV, 2018*



Minimal information for

file:///E:/学术盘/ISEV/Minimal%20information%20for%20studies%20of%20extracellular%20vesicles%202018.pdf

Taylor & Francis

Journal of Extracellular Vesicles

ISSN: (Print) 2001-3078 (Online) Journal homepage: http://www.tandfonline.com/loi/zjev20

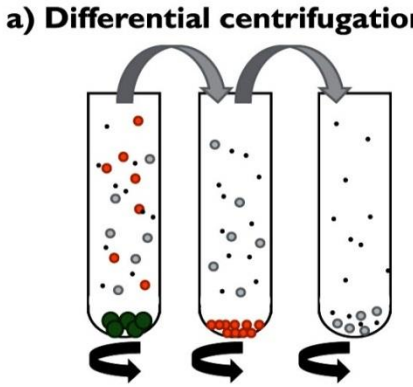
Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines

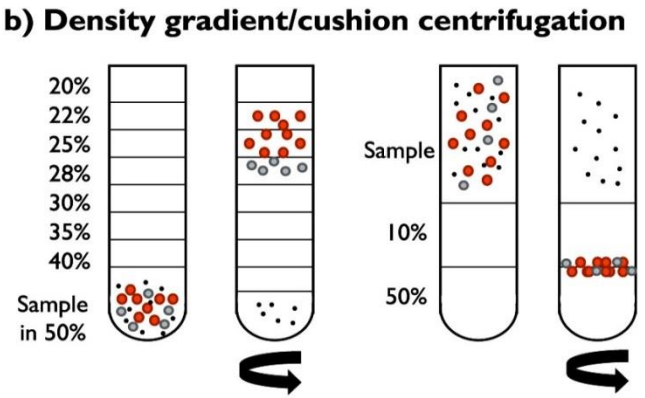
Clotilde Théry, Kenneth W Witwer, Elena Aikawa, Maria Jose Alcaraz, Johnathon D Anderson, Ramaroson Andriantsitohaina, Anna Antoniou, Tanina Arab, Fabienne Archer, Georgia K Atkin-Smith, D Craig Ayre, Jean-Marie Bach, Daniel Bachurski, Hossein Baharvand, Leonora Balaj, Shawn Baldacchino, Natalie N Bauer, Amy A Baxter, Mary Bebawy, Carla Beckham, Apolonija Bedina Zavec, Abderrahim Benmoussa, Anna C Berardi, Paolo Bergese, Ewa Bielska, Cherie Blenkiron, Sylwia Bobis-Wozowicz, Eric Boilard, Wilfrid Boireau, Antonella Bongiovanni, Francesc E Borràs, Steffi Bosch, Chantal M Boulanger, Xandra Breakefield, Andrew M Breglio, Meadhbh Á Brennan, David R Brigstock, Alain Brisson, Marike LD Broekman, Jacqueline F Bromberg, Paulina Bryl-Górecka, Shilpa Buch, Amy H Buck, Dylan Burger, Sara Busatto, Dominik Buschmann, Benedetta Bussolati, Edit I Buzás, James Bryan Byrd, Giovanni Camussi, David RF Carter, Sarah Caruso, Lawrence W Chamley, Yu-Ting Chang, Amrita Datta Chaudhuri, Chihchen Chen, Shuai Chen, Lesley Cheng, Andrew R Chin, Aled Clayton, Stefano P Clerici, Alex Cocks, Emanuele Cocucci, Robert J Coffey, Anabela Cordeiro-da-Silva, Yvonne Couch, Frank AW Coumans, Beth Coyle, Rossella Crescitelli, Miria Ferreira Criado, Crislyn D'Souza-Schorey, Saumya Das, Paola de Candia, Eliezer F De Santana Junior, Olivier De Wever, Hernando A del Portillo, Tanguy

22:21
2018-12-6

1. EVs 的分离与纯化常用技术

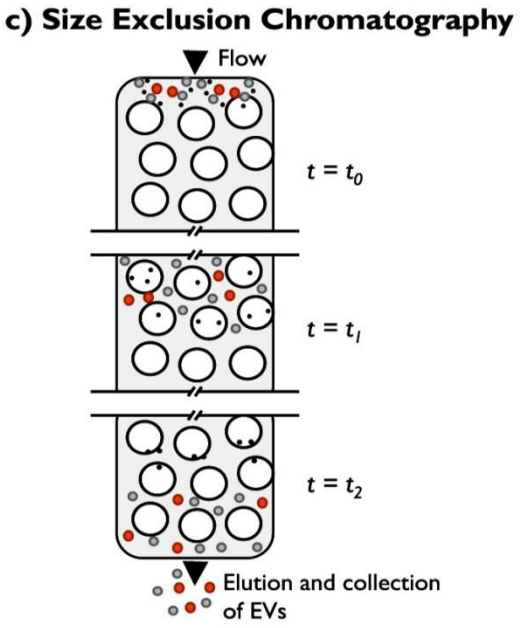
差速离心

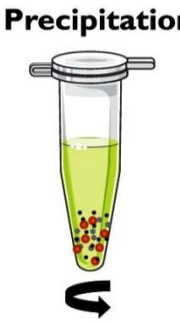




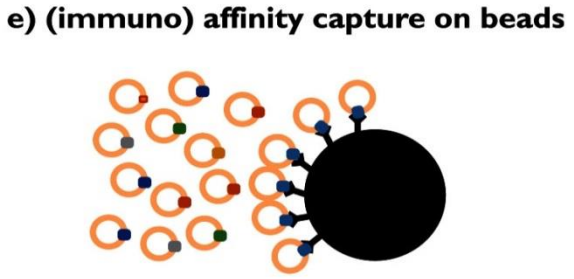
梯度密度离心

尺寸排阻





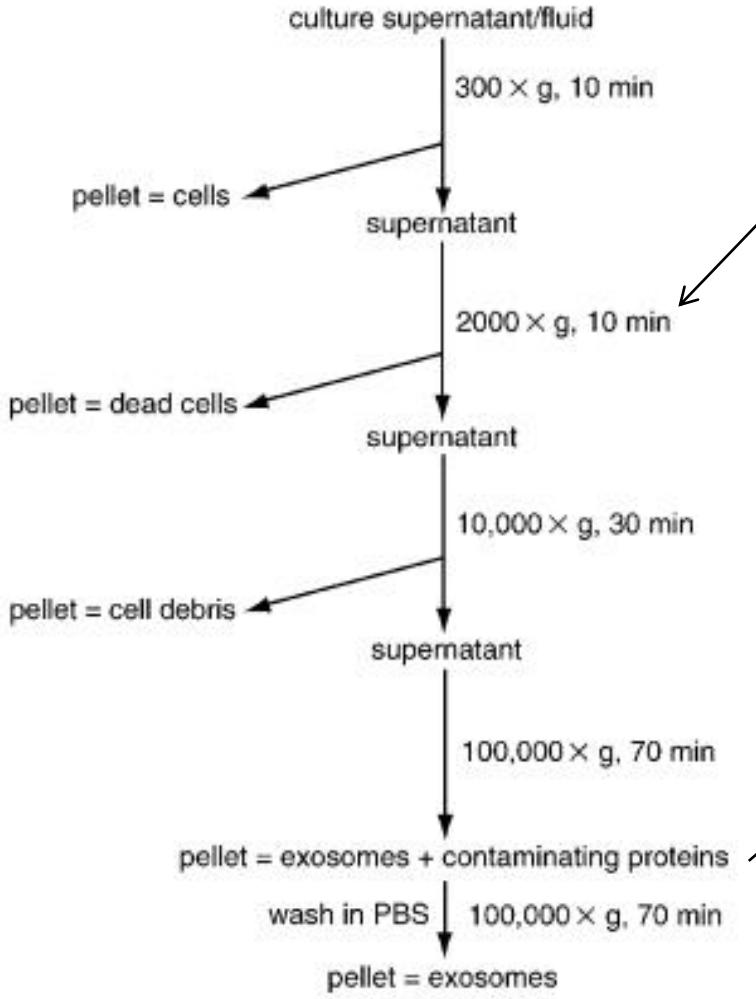
化学沉降



磁珠捕获

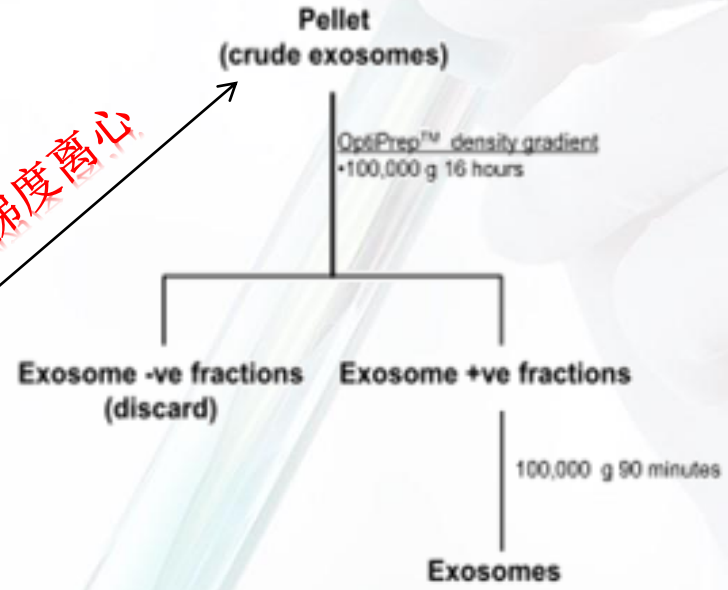
Bogdan Mateescu et al, Journal of Extracellular Vesicles (2017)

经典分离方法--超速离心法/蔗糖梯度离心



plasma/serum was diluted in the same or larger volume of PBS

蔗糖梯度离心



磁珠捕获

超滤法

CCM

300 x g,10min

pellet=cells

supematant

2000 x g,20min

pellet=dead cells

supematant

10000x g,30min

pellet=cell debris

supematant

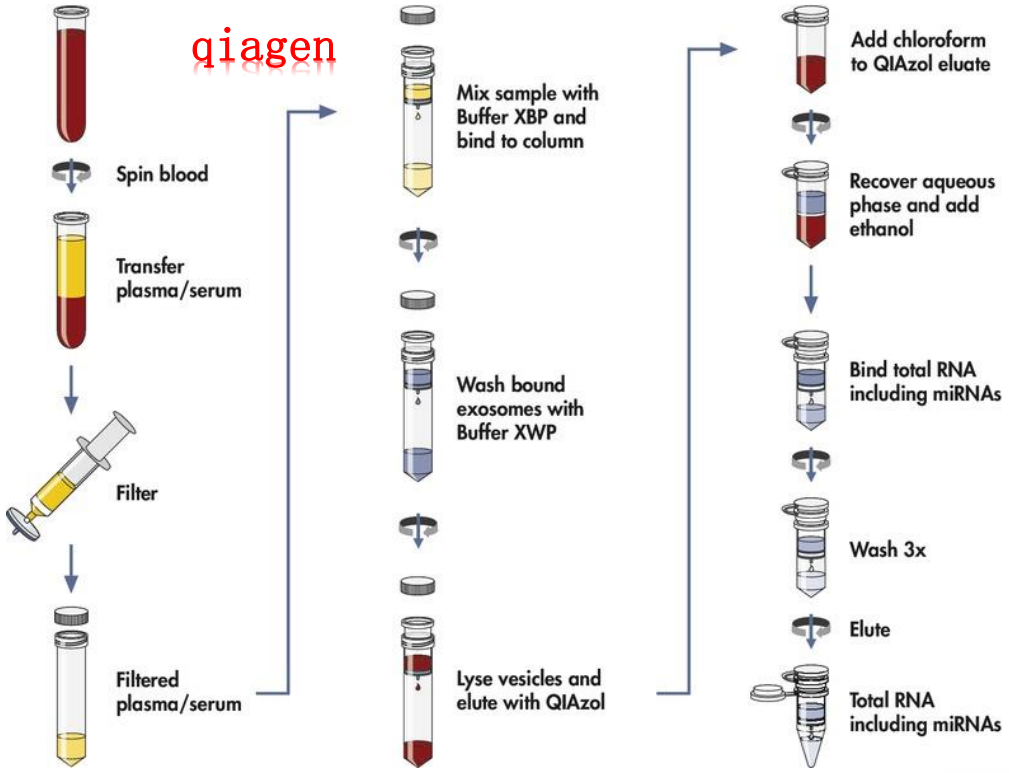
concentrate with Centricon

concentrate

磁珠捕获技术

新型试剂盒

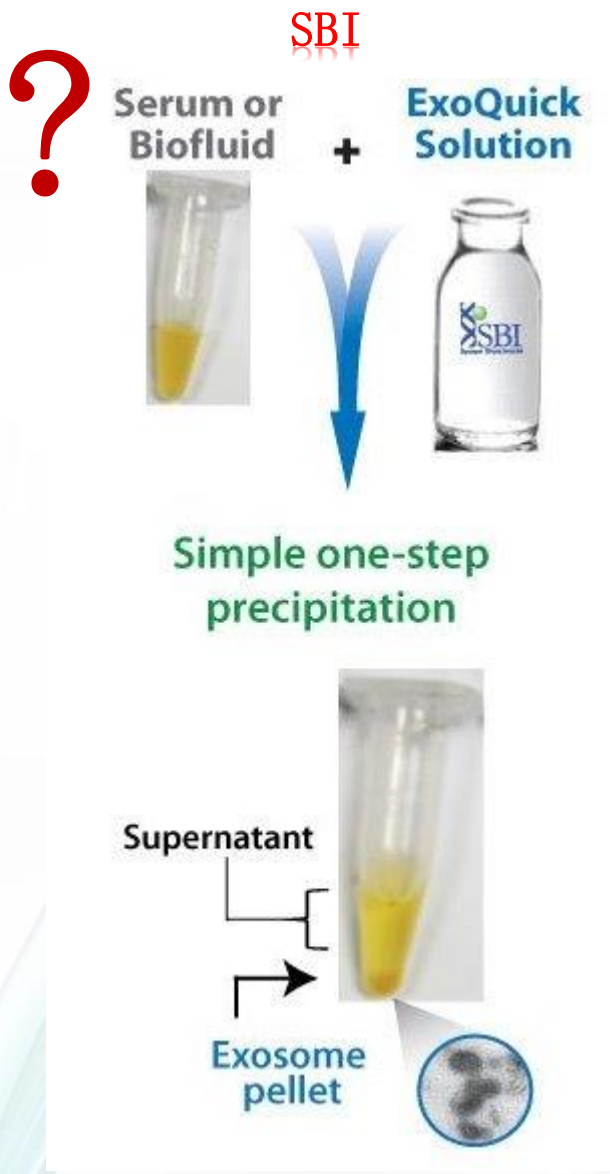
qiagen



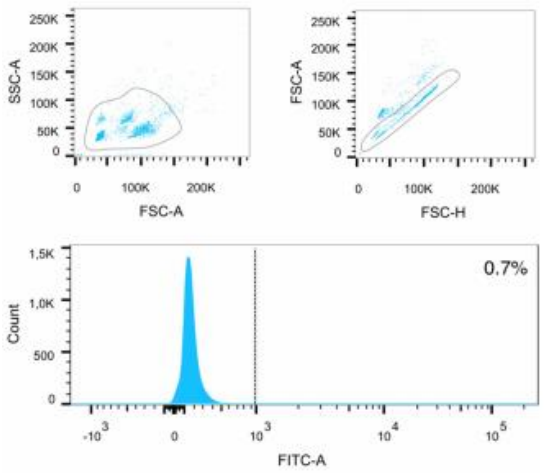
qEV

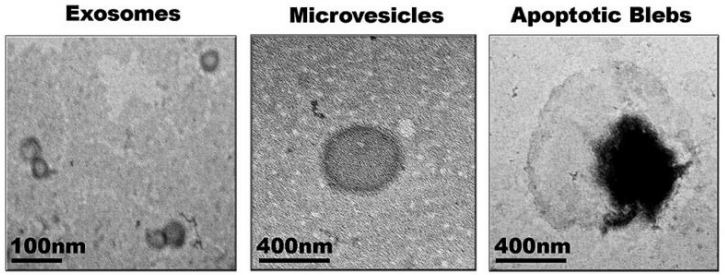
- ~15 minute separation time
- Greatly reduced risk of protein complex formation and vesicle aggregation Buffers with physiological osmolarity and viscosity can be used
- A gentle, rapid method for maximising recovery of biological function
- Vesicle recovery is expected to be 50% or greater
- Protein removal ratio > 1000
- HDL purification > 8 fold

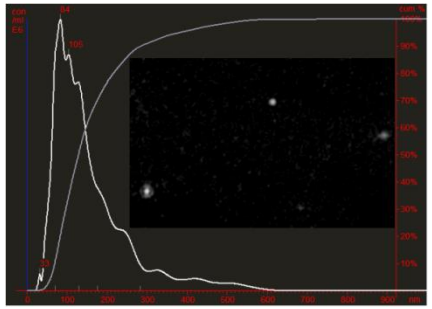
SBI

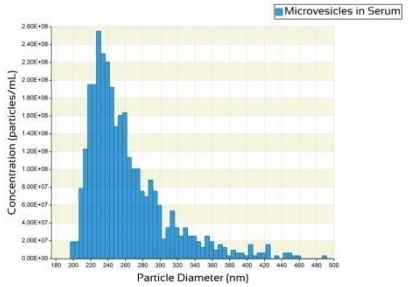


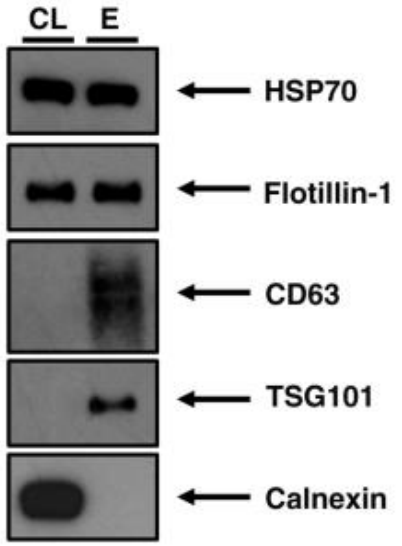
2.EVs的经典鉴定方法











3. EVs内容物分析

核酸

高通量测序技术
转录组学分析
荧光实时定量PCR
基因芯片
数字PCR

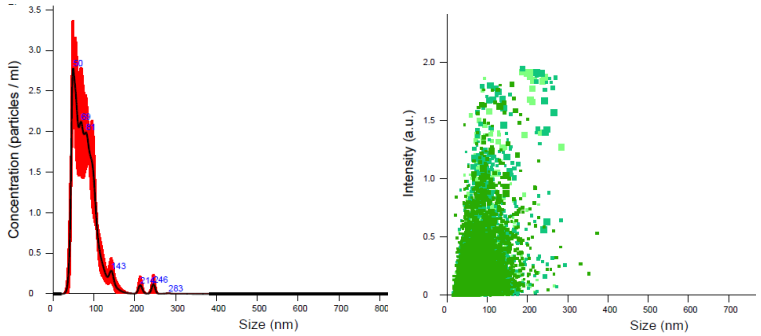
脂类

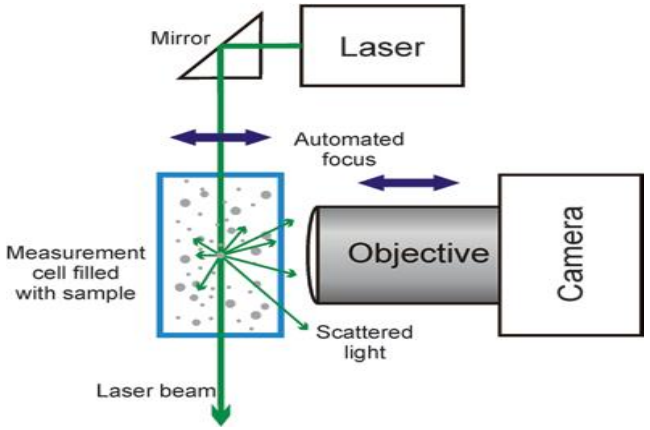
质谱分析
薄层色谱法

蛋白质

蛋白质组学分析
（质谱，2-DE等）
流式细胞术
ELISA
Western Blot
微型核磁共振系统

4. EVs 检测技术--粒径分布及数量检测





NTA技术可以清晰观察到带有散射光的颗粒的布朗运动，得到EVs浓度大小及粒径分布范围

Q-Nano技术具有样品用量少、尺寸小、可便携等特点，可实现快速，准确的计数和测量纳米微米颗粒的浓度

EVs 检测技术--多组学标志物检测

Mass Array分子量阵列基因分析

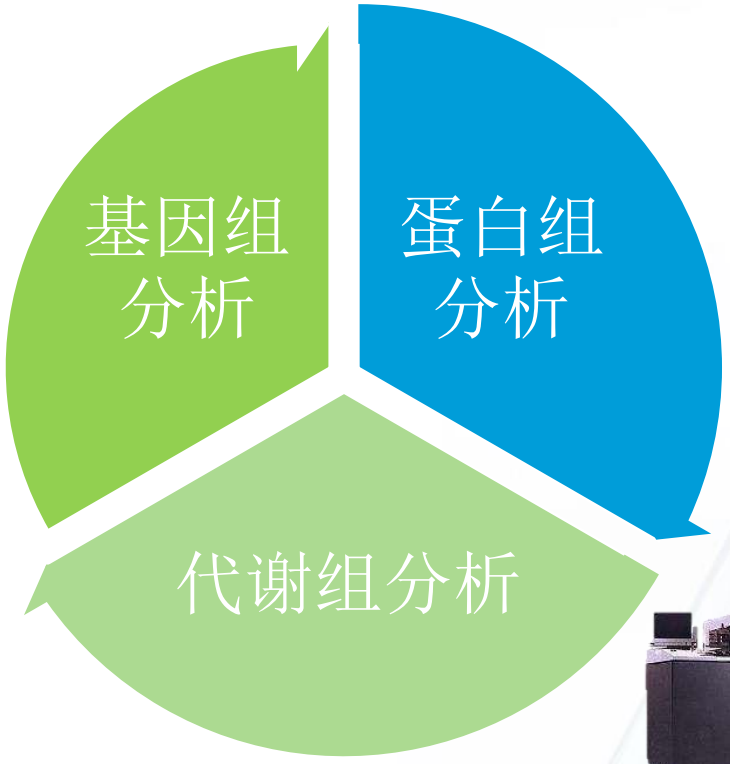
QToF MS/Orbit 飞行时间/轨道离子阱分析器

GC-MS 气相色谱-串联质谱

MALDI-ToF MS 基质辅助激光解吸电离飞行时间质谱

ICP-MS电感耦合等离子体质谱

LC-MS/MS 液相色谱-串联质谱



多组学整合分析

EVs 检测技术--多组学标志物的检测

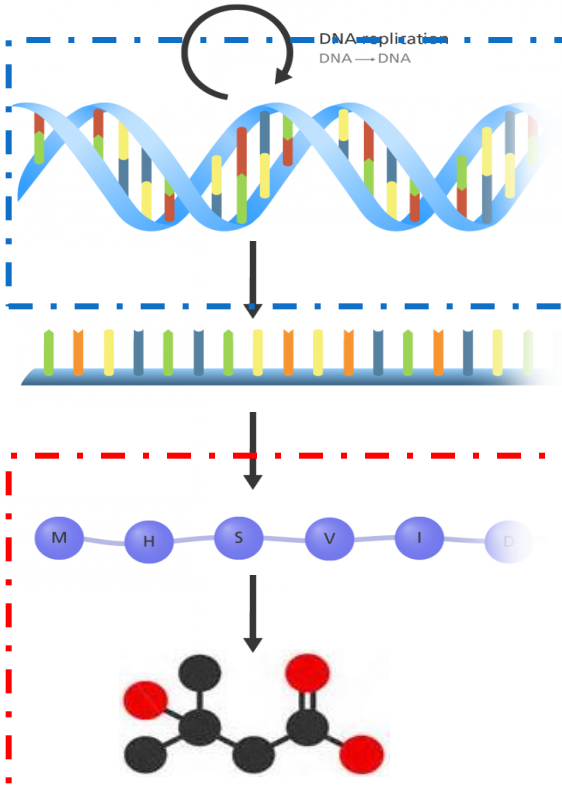
对可能发生事件的预测		通过代谢物信息库提示“已经发生了什么”
基因组学	VS	**蛋白质组和代谢组学**
通过基因分析对疾病进行风险评估		疾病已经发生，各种代谢物已经形成

研究蛋白质组学和代谢组学比基因组学更重要也更困难！
质谱可以解决！

EVs 检测技术--蛋白及代谢组标志物检测

基因是静止的，蛋白质和代谢物是动态的！
基因是有限的，蛋白质和代谢物是无限的！

质谱技术为海量级的蛋白组学和代谢组学研究提供技术支撑！



基因组
25,000 基因
Possibility

转录组
100,000 转录本
Probability

蛋白质组
1,000,000 蛋白质
What's happening

代谢组
2,500 化合物
What happened

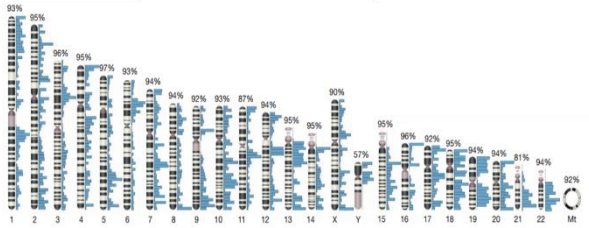
二代测序

质谱

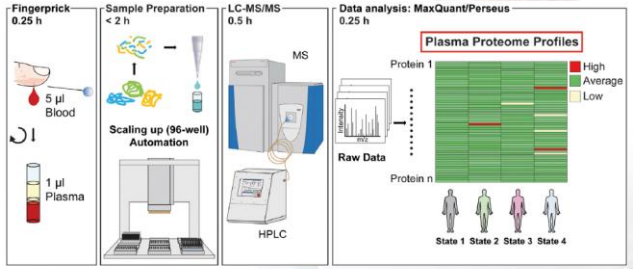
EVs 检测技术--蛋白及代谢组标志物检测

蛋白质组

- 人类蛋白质组草图(2014)：17,294 种 蛋白质（84% 基因覆盖）

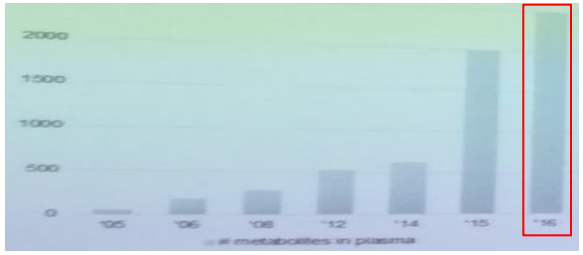


- 血浆：>1000种蛋白；组织：>10,000种蛋白
- 采用质谱技术，3小时从采血到出报告

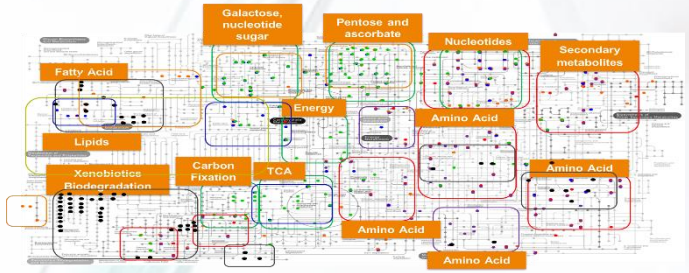


代谢组

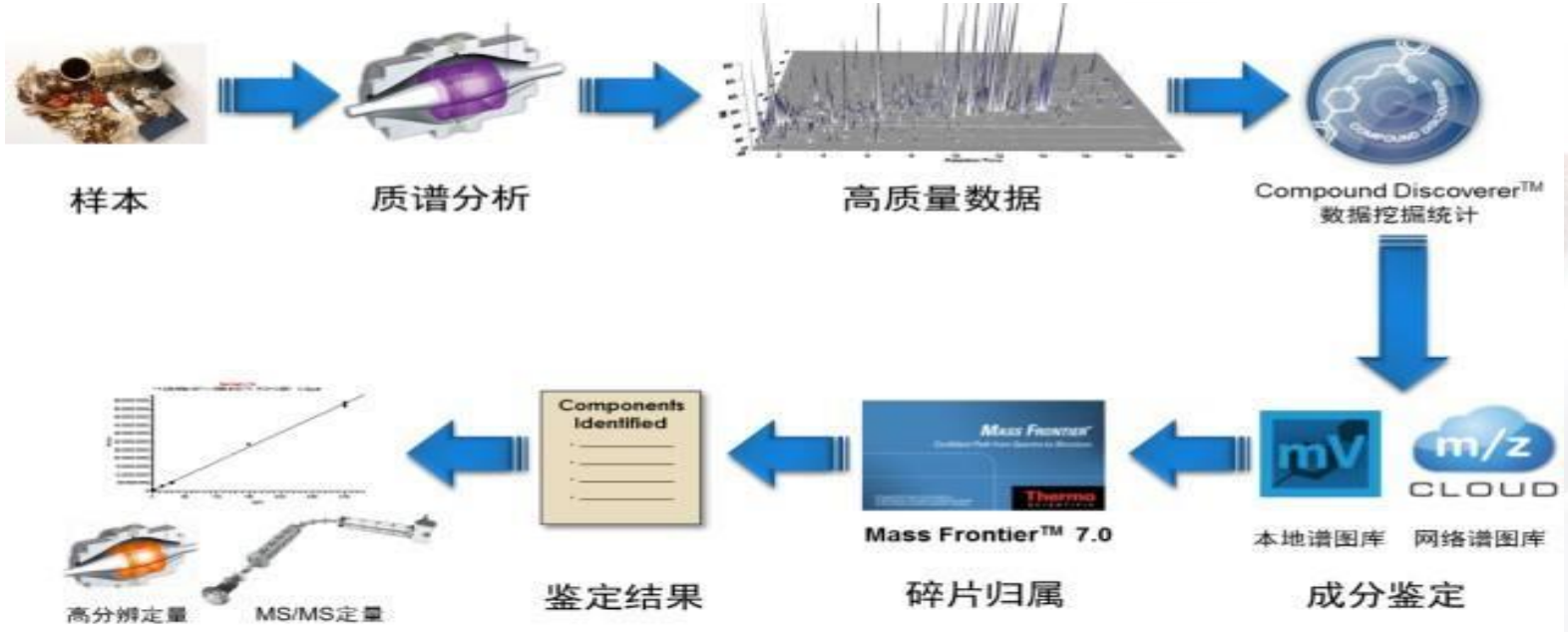
- 分析深度达到2500种化合物，10年提高一个数量级



- 一个血浆样本近1000种代谢物。这些代谢物变化和疾病相关。常规测定技术困难，质谱可以解决！



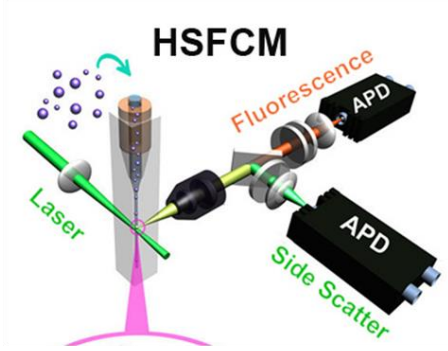
EVs 检测技术--蛋白及代谢组标志物的检测

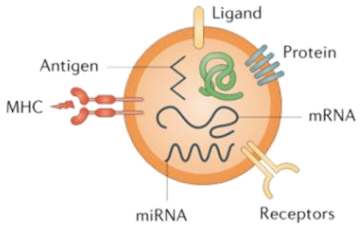


超高分辨质谱卓越的灵敏度和准确性可很好地应对外泌体低丰度生物标志物检测的挑战！

EVs 检测技术--特定蛋白标志物的检测

纳米流式检测仪



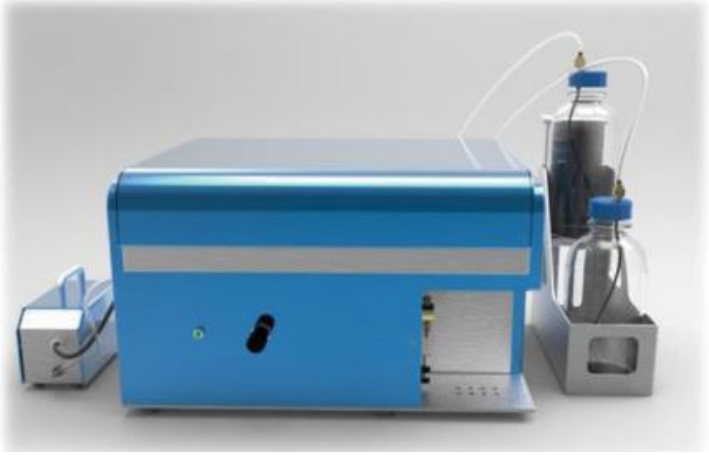


Size

Particle Concentration

Biochemical properties

- 特定亚群的鉴定
- 疾病诊断和治疗监控
- 外泌体纯化方法的分析表征
- EV相关产品的质量控制
- ……



SSC Sensitivity	< 30 nm NPs
SSC Resolution	40/50 nm NPs
Fluorescence Sensitivity	<10 MESF
Fluorescence Resolution	42/133 MESF
Particle Size	7-500 nm
Sample Acquisition Rate	10,000 events/min

EVs 检测技术—核酸标志物的检测

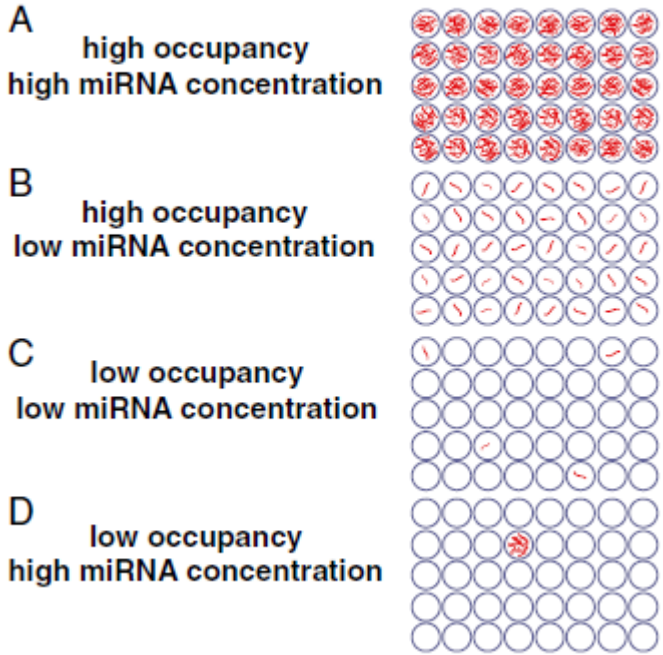
数字PCR技术

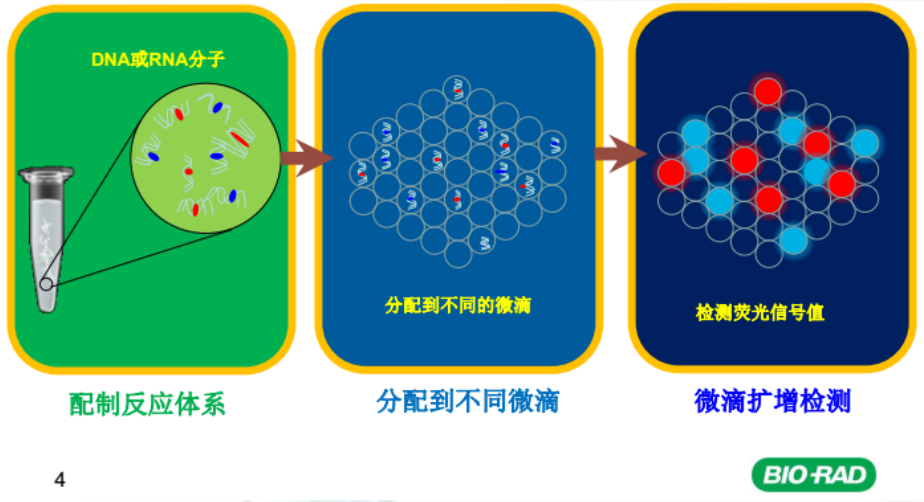
CrossMark click for updates

Quantitative and stoichiometric analysis of the microRNA content of exosomes

John R. Chevillet[a], Qing Kang[a,b], Ingrid K. Ruf[a,1], Hilary A. Briggs[a,1], Lucia N. Vojtech[c,1], Sean M. Hughes[c,1], Heather H. Cheng[a,d], Jason D. Arroyo[a], Emily K. Meredith[a], Emily N. Gallichotte[a], Era L. Pogosova-Agadjanyan[e], Colm Morrissey[f], Derek L. Stirewalt[e], Florian Hladik[c,d,g], Evan Y. Yu[d], Celestia S. Higano[d,e,f], and Muneesh Tewari[a,b,e,h,i,j,k,2]

Divisions of [a]Human Biology, [c]Clinical Research, [g]Vaccine and Infectious Disease, and [i]Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; Departments of [b]Internal Medicine and [h]Biomedical Engineering, [j]Biointerfaces Institute, and [k]Center for Computational Medicine, University of Michigan, Ann Arbor, MI 48109; and Departments of [e]Obstetrics and Gynecology, [d]Medicine, and [f]Urology, Division of Oncology, University of Washington, Seattle, WA 98195

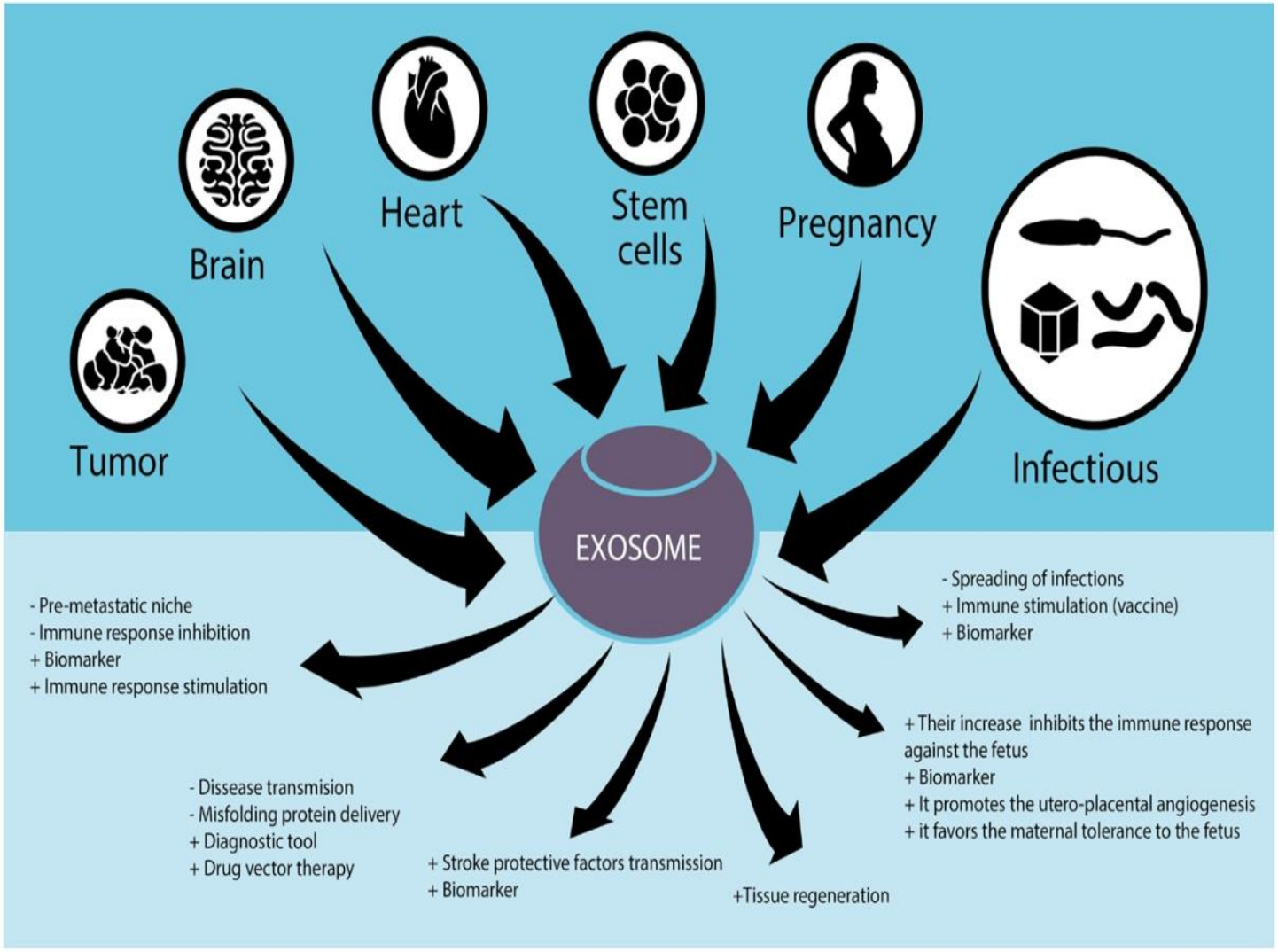




Chevillet John R et al.Proc. Natl. Acad. Sci. U.S.A., 2014, 111(41): 14888-93.

2. EVs用于疾病诊断

EVs与疾病诊断


Brain
Heart
Stem cells
Pregnancy
Tumor
Infectious
EXOSOME
- Pre-metastatic niche
- Immune response inhibition
+ Biomarker
+ Immune response stimulation
- Spreading of infections
+ Immune stimulation (vaccine)
+ Biomarker
- Dissease transmision
- Misfolding protein delivery
+ Diagnostic tool
+ Drug vector therapy
+ Stroke protective factors transmission
+ Biomarker
+Tissue regeneration
+ Their increase inhibits the immune response against the fetus
+ Biomarker
+ It promotes the utero-placental angiogenesis
+ it favors the maternal tolerance to the fetus

1.EVs作为诊断标志物的优势

- 无创，有望作为病情监测标志物；
- 更全面的反应肿瘤情况；
- 灵敏度高且血清干扰较少；
- 存在于多种体液中；
- 脂质双层膜保护作用，分子更稳定，易于保存

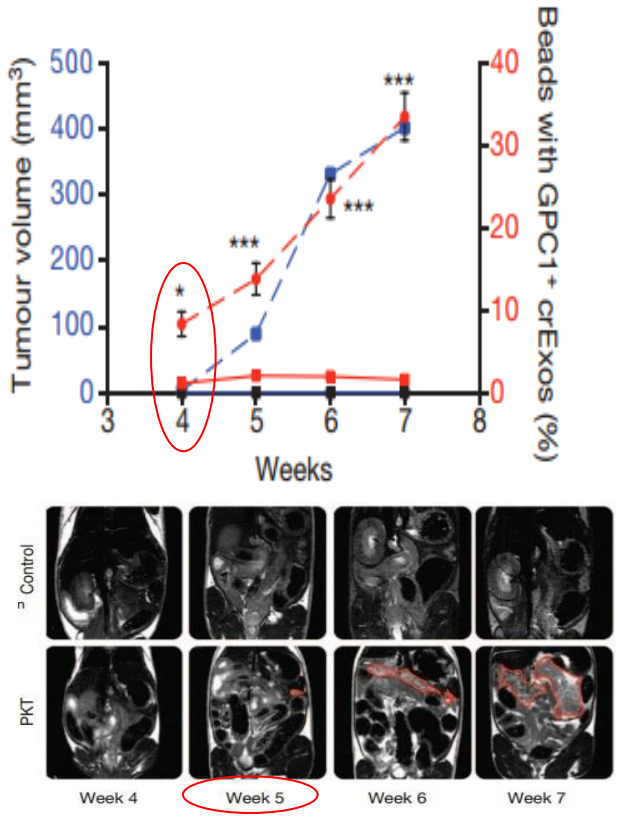
2. EVs诊断标志物

EVs biomarkers

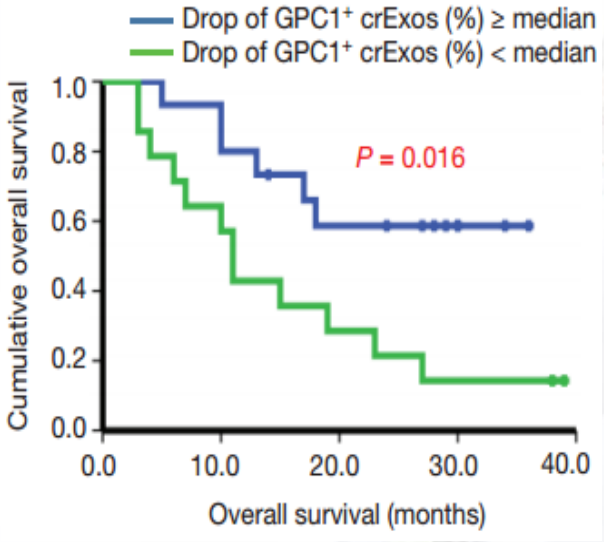
- DNA
- RNA
- Lipids
- Proteins
- Peptide
- Metabolites
- Glycan, ect.

EVs中蛋白质作为诊断标志物

GPC1+exosome用于诊断早期胰腺癌



胰腺癌小鼠模型血液exosome GPC 1升高早于影像学。

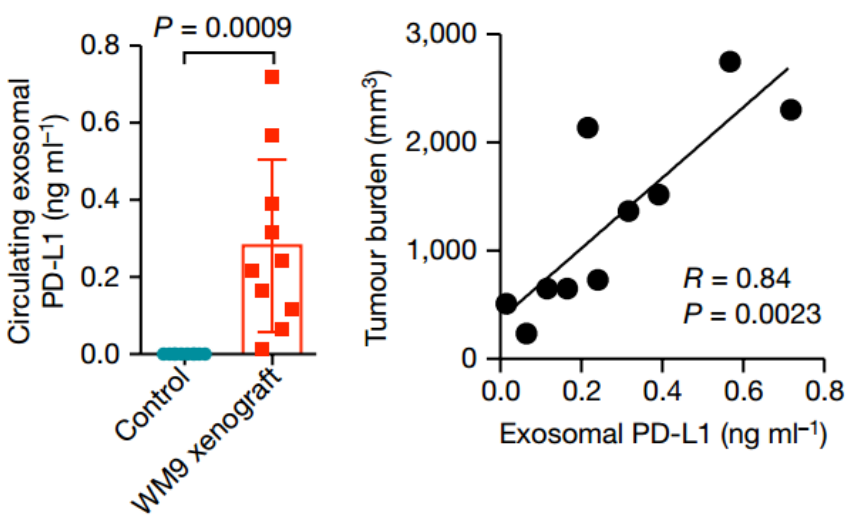


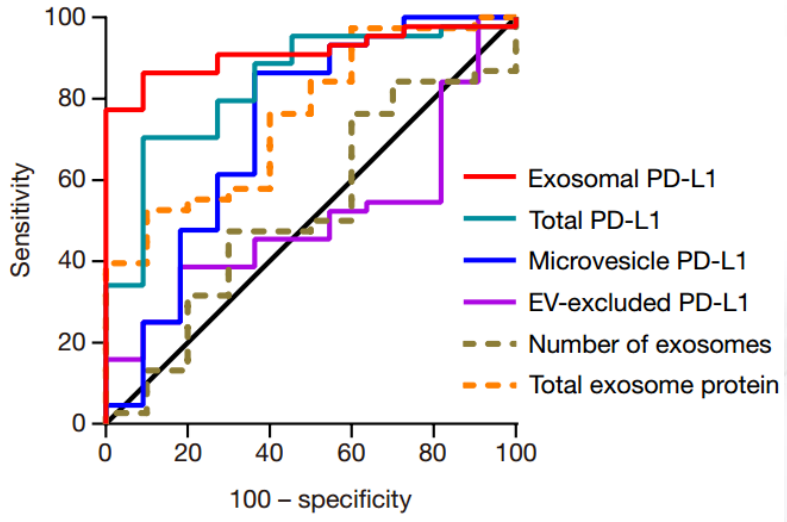
胰腺癌患者手术后，GPC1+exosome下降多，患者生存率高。

Nature. 2015 Jul 9;523(7559):177-82.

EVs中蛋白质作为治疗效果标志物

血液循环Exosomal PD-L1用于区分健康人和黑色素瘤病人





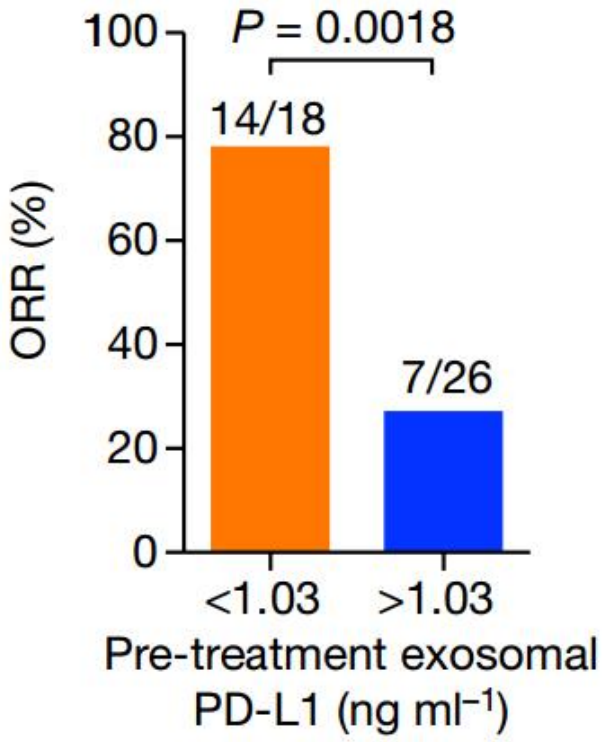
黑色素瘤小鼠血浆外泌体中PD-L1分子水平明显上调，且与肿瘤体积大小成正相关。

外泌体PD-L1分子的水平最适合用于区分健康人和黑色素瘤病人。

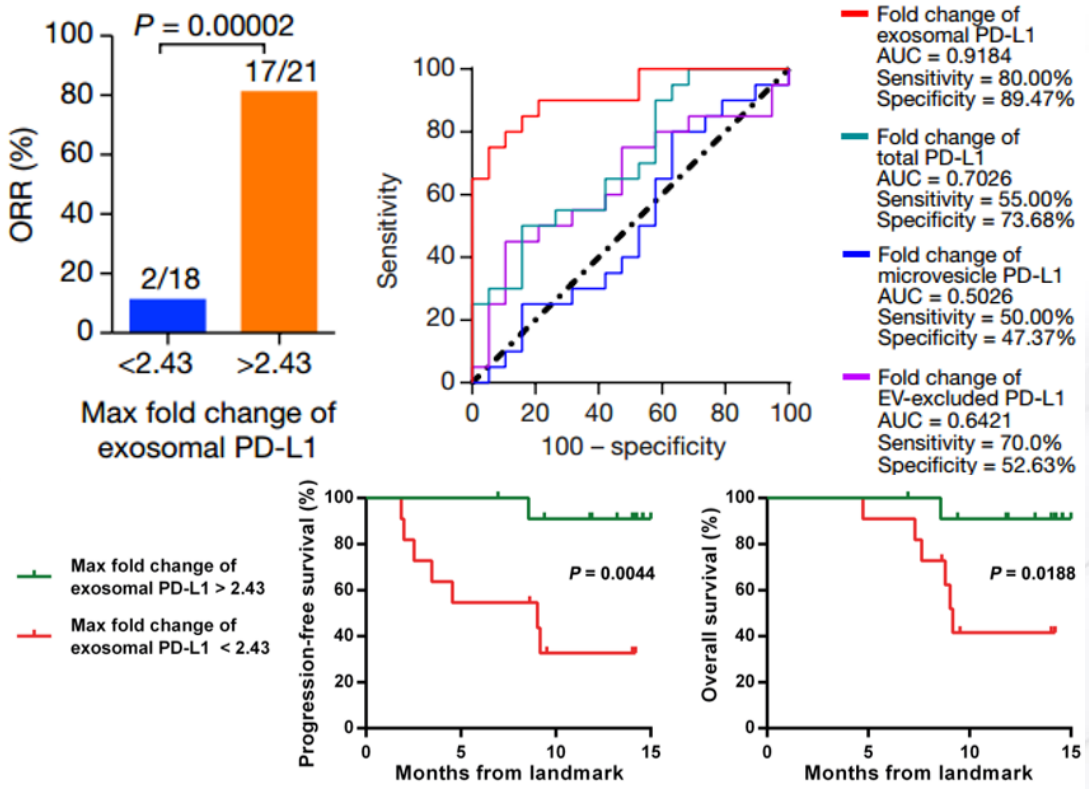
Nature. 2018 Aug;560(7718):382-386

EVs中蛋白质作为治疗效果标志物

血液循环Exosomal PD-L1用于指导临床用药和预测临床预后



治疗前期，血浆中的外泌体PD-L1水平越高，药物响应越差，预后越差。



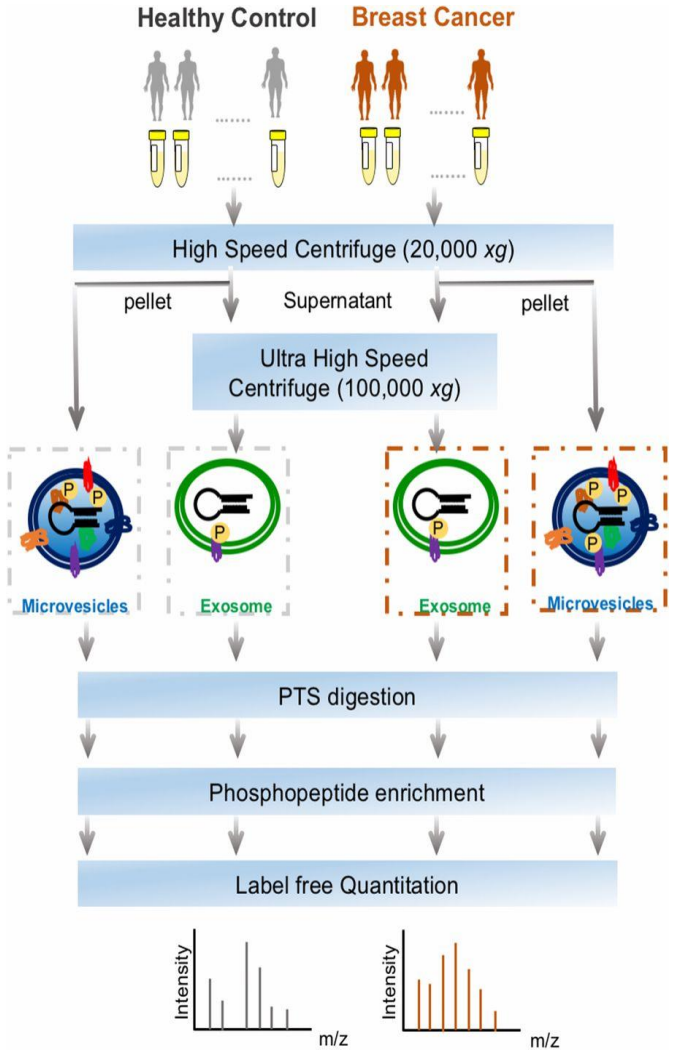
第3-6周，外泌体PD-L1水平的倍数增加大于2.43时，可更好地区分治疗响应好和坏的患者。

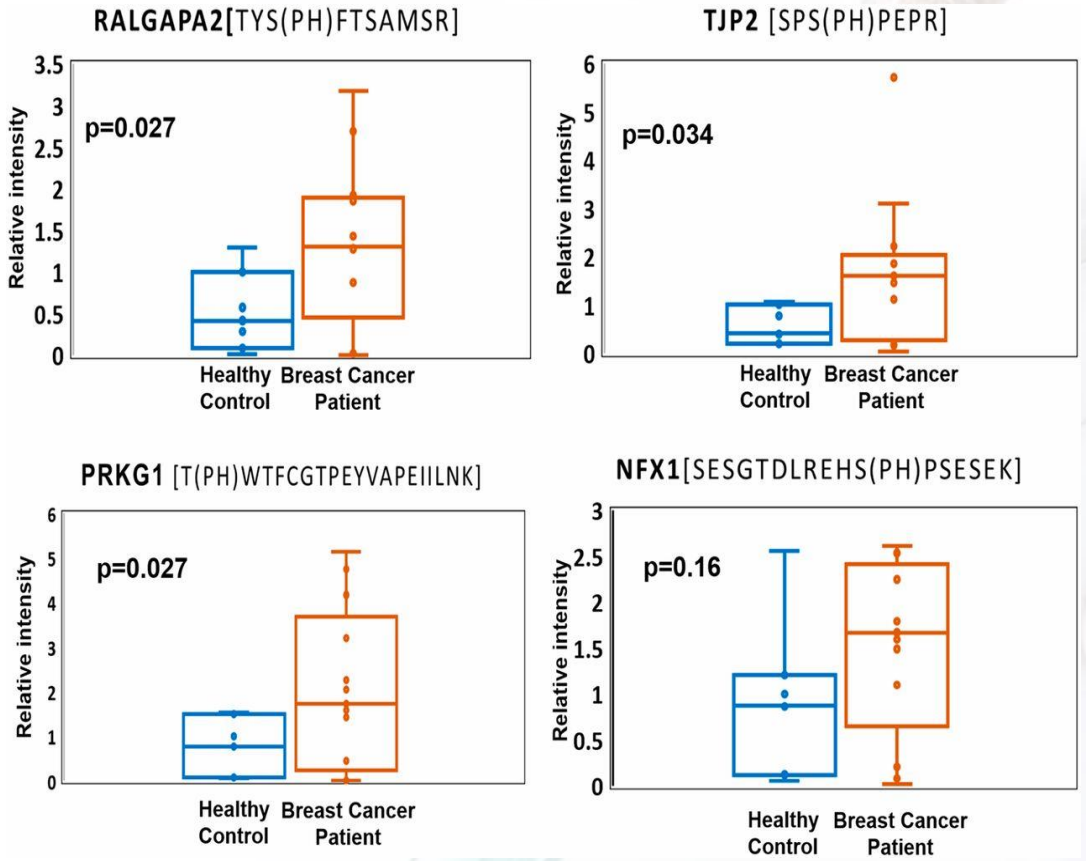
Nature. 2018 Aug;560(7718):382-386

PTMs of proteins in EVs as biomarkers

Phosphoproteins in extracellular vesicles as candidate markers for breast cancer

I-Hsuan Chen[a], Liang Xue[a], Chuan-Chih Hsu[a], Juan Sebastian Paez Paez[a], Li Pan[b], Hillary Andaluz[c], Michael K. Wendt[b], Anton B. Iliuk[d], Jian-Kang Zhu[a,e,f,1], and W. Andy Tao[a,b,c,g,1]



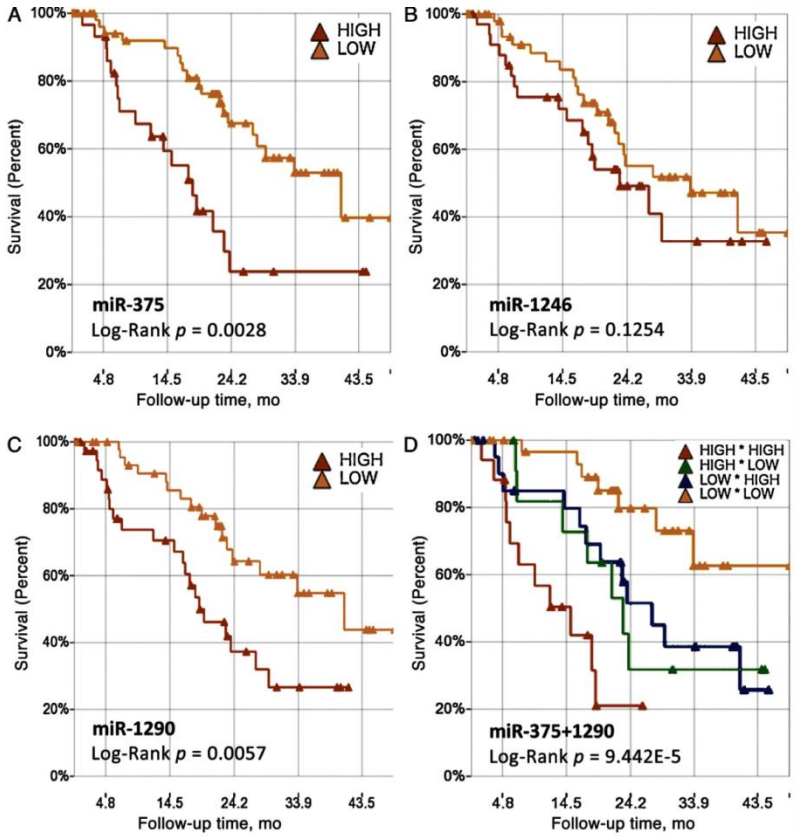


Proc Natl Acad Sci U S A. 2017 Mar 21;114(12):3175-3180

EVs中核酸作为诊断标志物

Exosomal miR-1290 and miR-375 as Prognostic Markers in Castration-resistant Prostate Cancer

Xiaoyi Huang[a,b], Tiezheng Yuan[a], Meihua Liang[c], Meijun Du[a], Shu Xia[a,d], Rachel Dittmar[a], Dian Wang[e], William See[f], Brian A. Costello[g], Fernando Quevedo[g], Winston Tan[h], Debashis Nandy[g], Graham H. Bevan[i], Sherri Longenbach[g], Zhifu Sun[j], Yan Lu[k], Tao Wang[l], Stephen N. Thibodeau[m], Lisa Boardman[g], Manish Kohli[g,], Liang Wang[a,*]*

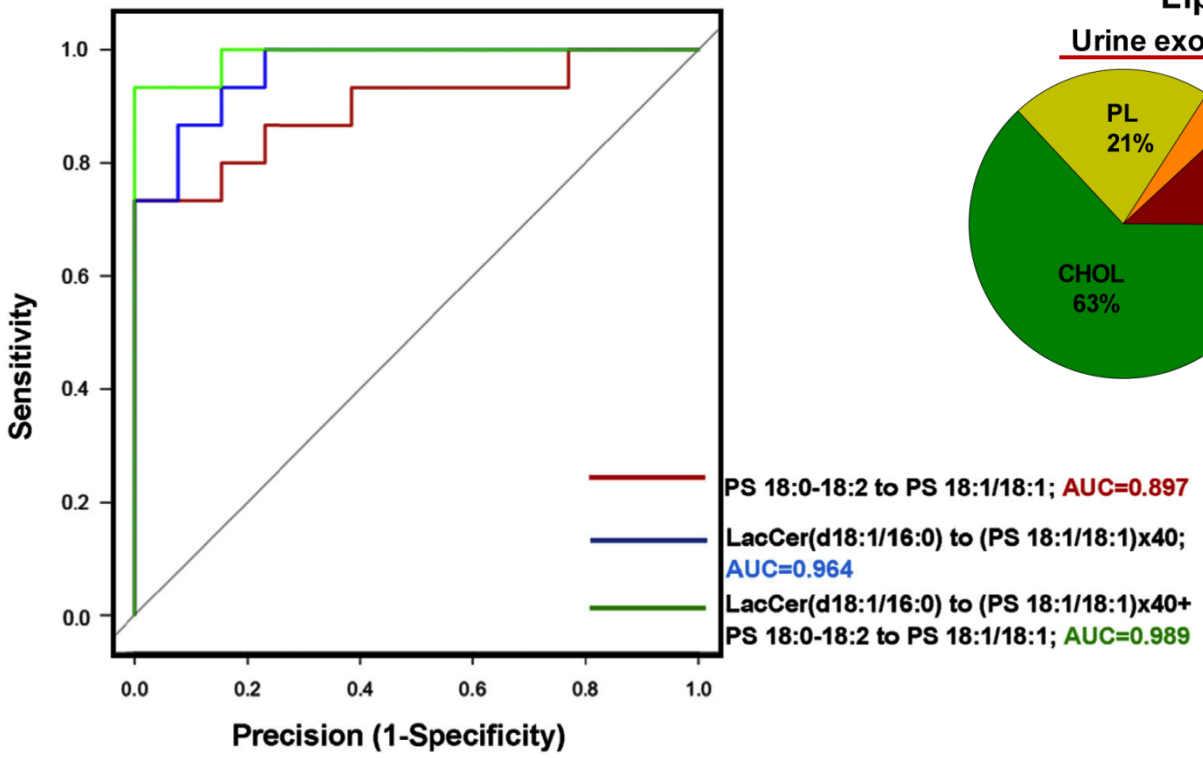


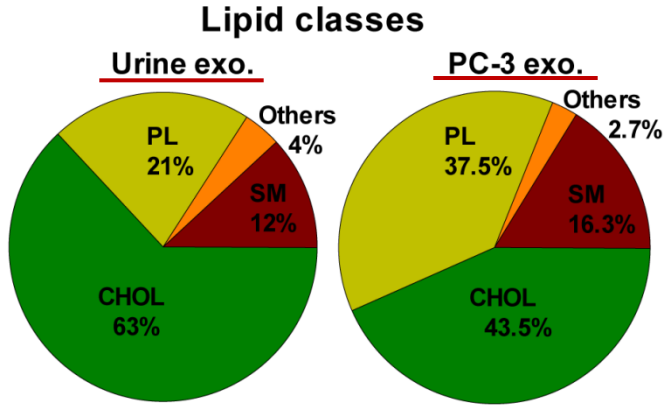
Higher levels of miR-1290 and -375 were significantly associated with poor overall survival ($p < 0.004$) in the follow-up cohort.

Xiaoyi Huang et al, European Urology (2015)

EVs中脂类作为诊断标志物

尿液exosome中乳糖神经酰胺（LacCer）和磷脂酰丝氨酸（PS）用于前列腺癌检测

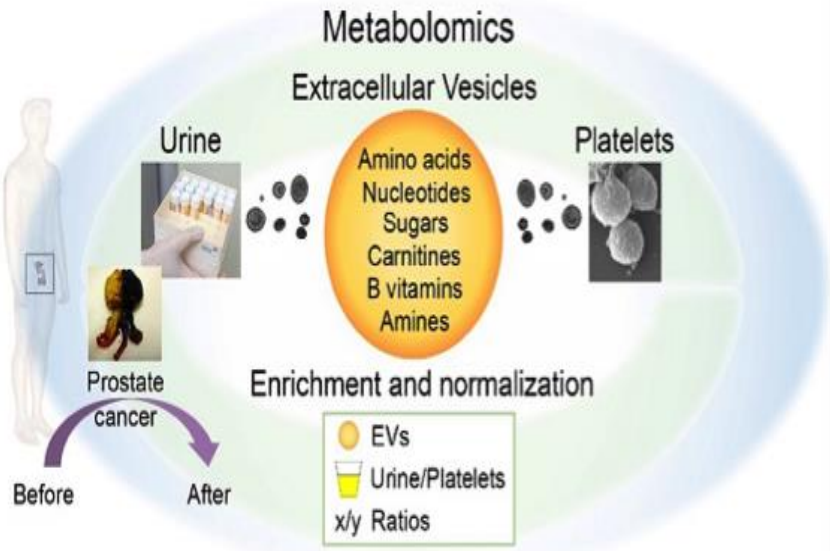


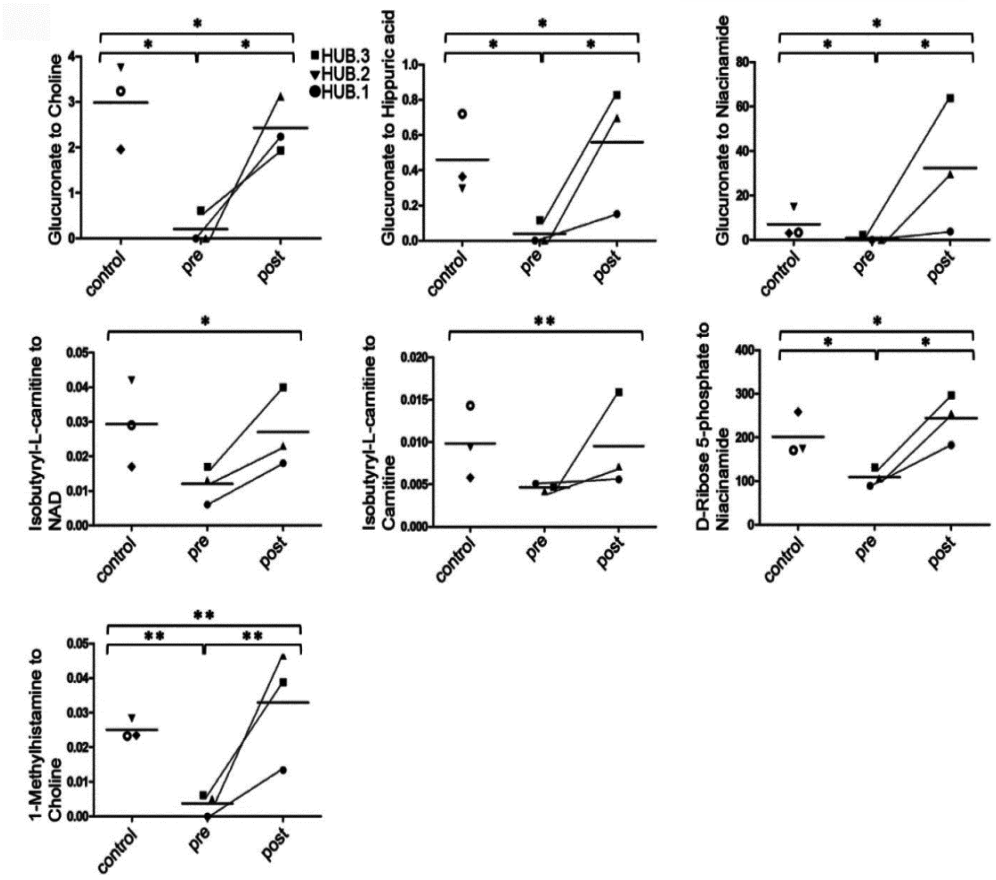


Skotland T et al, European Journal of Cancer (2017)

EVs中代谢物作为诊断标志物

Metabolomic Profiling of Extracellular Vesicles and Alternative Normalization Methods Reveal Enriched Metabolites and Strategies to Study Prostate Cancer-Related Changes





Glucuronate, etc

Theranostics. 2017 Aug 23;7(16):3824-3841.

NIH注册的exosome诊断试剂临床试验

Table 1. Current or Recently Completed Registered National Institutes of Health (NIH) Clinical Trials Involving Exosomes as Diagnostic Agents[a]

Study title	Disease	Study design	Start date	NCT[b]
Circulating Exosome as Potential Prognostic and Predictive Biomarkers in Advanced Gastric Cancer Patients: A Prospective Observational Study ('EXO-PPP Study')	Gastric cancer	Prospective trial observational phase not provided (currently recruiting)	Jan 2013	NCT01779583
An Observational, Single-Institution Pilot/Feasibility Study of Exosome Testing as a Screening Modality for Human Papillomavirus-Positive Oropharyngeal Squamous Cell Carcinoma	Oropharyngeal cancer	Prospective trial observational phase not provided (currently recruiting)	Feb 2015	NCT02147418
LRRK2 and Other Novel Exosome	Parkinson's	Prospective trial	Jan 2013	NCT01860118

Continue。。。。。

第一个基于外泌体液体活检的临床检测试剂盒

ExoDx®
Prostate(IntelliScore)

CLIA认证，临床可用

Original Investigation

A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy

JAMA Oncol. doi:10.1001/jamaoncol.2016.0097

1500余例标本多中心的研究中，
将高级别前列腺癌（GS≥7）与低级别前列腺癌（GS 6）及前列腺良性病变的鉴别诊断效能从0.63提高到0.73

收集尿液，提取外泌体，检测外泌体中3条RNA，代入诊断模型，评估前列腺癌风险

EVs诊断标志物筛选主要策略

EVs诊断标志物筛选主要策略

1. 细胞系；

2. 临床血液体液样本；

3. 患病组织；

4. 前期研究或文献

1. 细胞系样本

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2016

Fibronectin on circulating extracellular vesicles as a liquid biopsy to detect breast cancer

Pyong-Gon Moon[1], Jeong-Eun Lee[1], Young-Eun Cho[1], Soo Jung Lee[2], Yee Soo Chae[2], Jin Hyang Jung[3], In-San Kim[4], Ho Yong Park[3], Moon-Chang Baek[1]

[1]Department of Molecular Medicine, Cell and Matrix Research Institute, School of Medicine, Kyungpook National University, Daegu 700-422, Republic of Korea

[2]Department of Oncology/Hematology, Kyungpook National University Hospital, Daegu 700-721, Republic of Korea

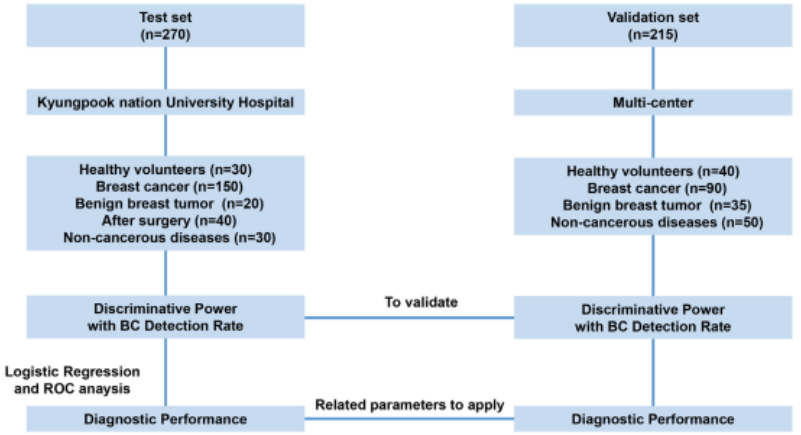
[3]Department of Breast & Thyroid Surgery, Kyungpook National University Hospital, Daegu 700-721, Republic of Korea

[4]Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, KU-KIST School, Korea University, Seoul 02841, Republic of Korea

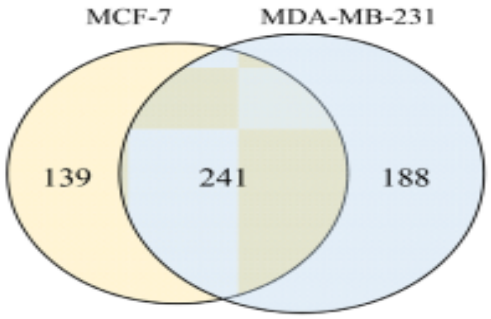
Correspondence to: *Moon-Chang Baek,* ***email:*** *mcbaek@knu.ac.kr*

Keywords: *breast cancer, diagnosis, extracellular vesicle, ELISA*

Received: *September 15, 2015* ***Accepted:*** *May 02, 2016* ***Published:*** *May 23, 2016*



A



B

Accession	Protein Description
IPI00022418	Fibronectin
IPI00095891	GNAS complex locus
IPI00220834	ATP-dependent DNA helicase 2 subunit 2
IPI00644712	ATP-dependent DNA helicase 2 subunit 1
IPI00013468	Isoform 1 of Mitotic checkpoint protein BUB3
IPI00304925	Heat shock 70 kDa protein 1A/1B
IPI00911039	Heat shock 70 kDa protein 1
IPI00003865	Isofo
IPI00013744	Integ
IPI00215995	Isofo
IPI00218342	C-1-t
IPI00169383	Phos
IPI00002894	DNA
IPI00296337	Isofo
IPI00022462	Trans
IPI00013683	Tubu
IPI00645452	Tubu

	AUC (95%CI)	Sensitivity (%)	Specificity (%)	LR +	NR -
Method 1					
BC *vs* HC+bB+NC	0.748 (0.683-0.812)	68.9%	72.0%	2.43	0.44
BC *vs* bB+NC	0.736 (0.666-0.806)	66.1%	74.2%	2.56	0.46
Early-BC *vs* HC+bB+NC	0.737 (0.657-0.812)	67.7%	72.0%	2.42	0.45
Early- BC *vs* bB+NC	0.722 (0.637-0.807)	67.7%	74.3%	2.63	0.43
Method 2					
BC *vs* HC+bB+NC	0.684 (0.614-0.753)	54.4%	75.2%	2.19	0.61
BC *vs* bB+NC	0.665 (0.589-0.741)	56.0%	75.7%	2.30	0.58
Early-BC *vs* HC+bB+NC	0.672 (0.591-0.753)	49.2%	75.2%	1.98	0.68
Early- BC *vs* bB+NC	0.654 (0.566-0.743)	49.2%	76.7%	2.11	0.66

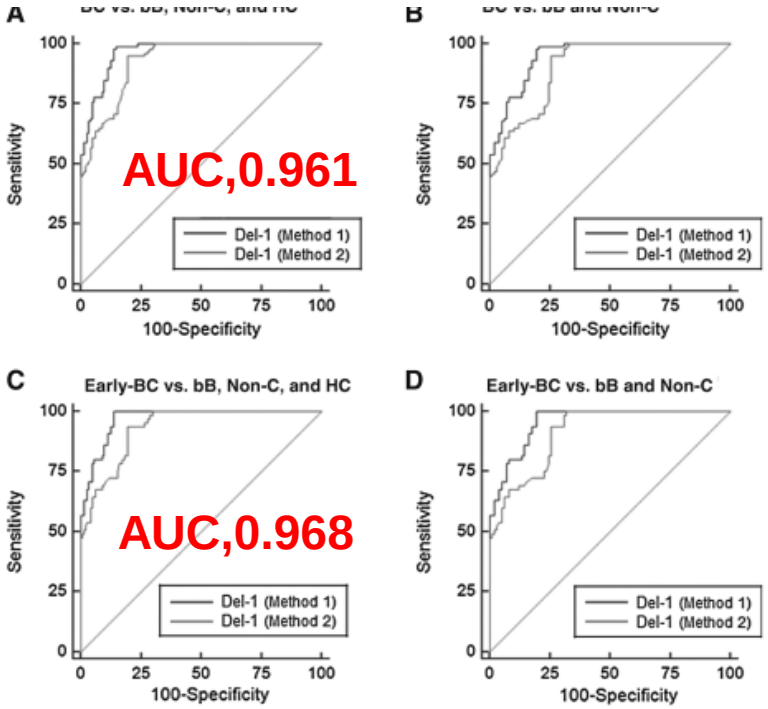
2. 临床血液体液样本

Biology of Human Tumors

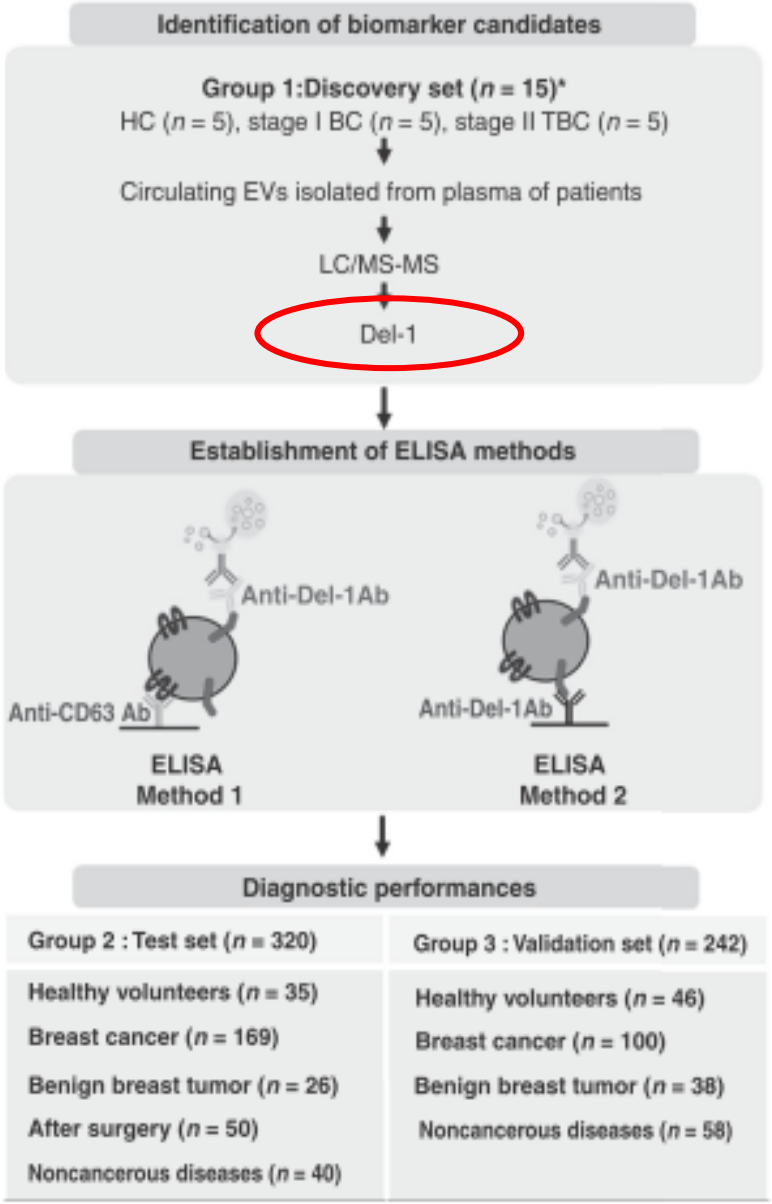
Clinical Cancer Research

Identification of Developmental Endothelial Locus-1 on Circulating Extracellular Vesicles as a Novel Biomarker for Early Breast Cancer Detection

Pyong-Gon Moon[1], Jeong-Eun Lee[1], Young-Eun Cho[1], Soo Jung Lee[2], Jin Hyang Jung[3], Yee Soo Chae[2], Han-Ik Bae[4], Young-Bum Kim[5], In-San Kim[6], Ho Yong Park[3], and Moon-Chang Baek[1]

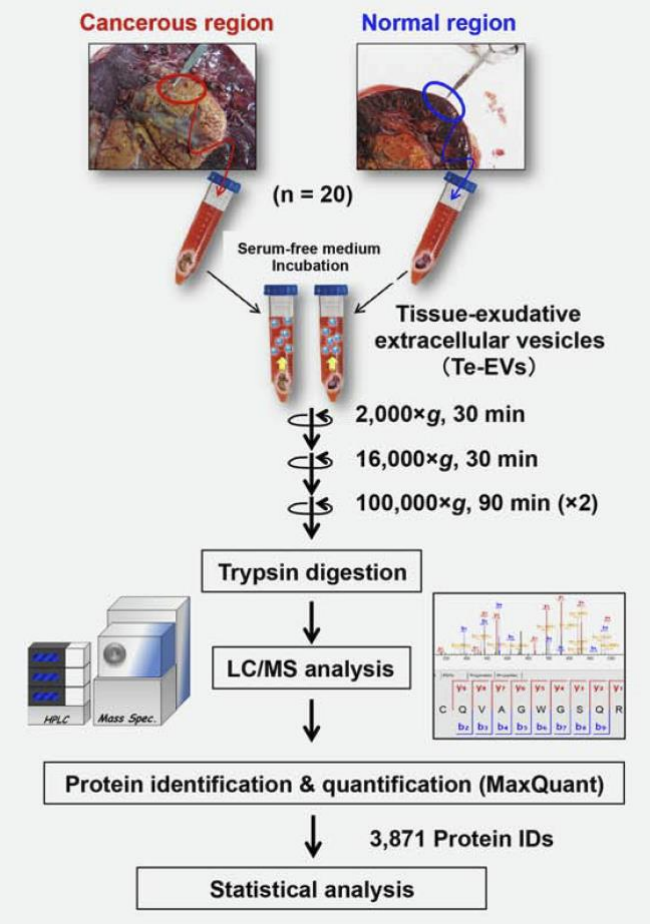


validation set



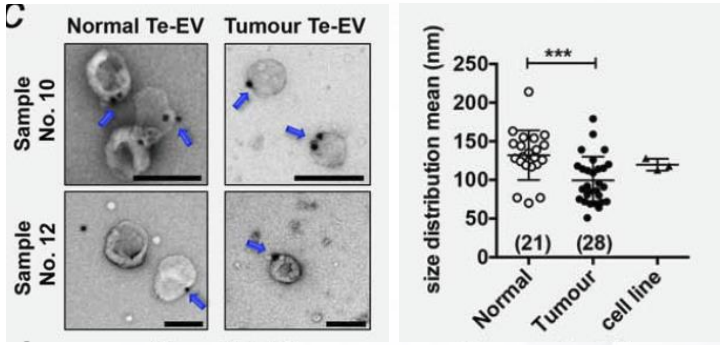
3. 临床患病组织

Extracellular vesicles isolated from human renal cell carcinoma



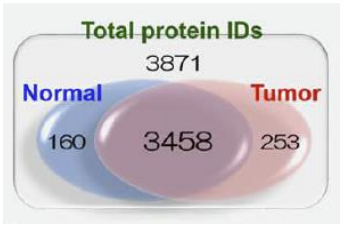
(Jingushi, Uemura et al. *Int J Cancer 2018*)

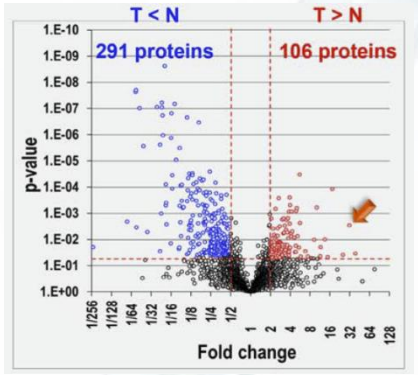
Tissue-exudative EVs (Te-EVs) characterization



IEM: Labeled with CD9

Differentially expressed proteins





AZU1
(P<0.01,
fold-change=31.59).

T: tumor, N: Normal

4. 前期研究或文献

nature
International journal of science

Article | Published: 28 October 2015

Tumour exosome integrins determine organotropic metastasis

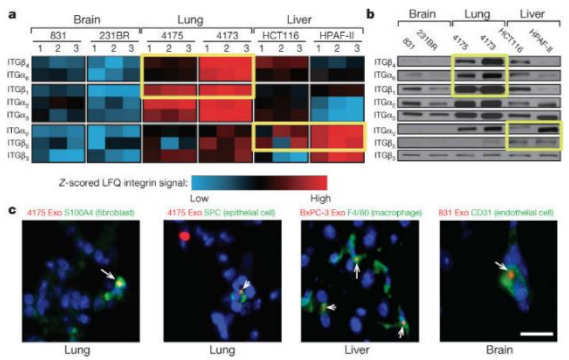
Ayuko Hoshino, Bruno Costa-Silva [...] David Lyden

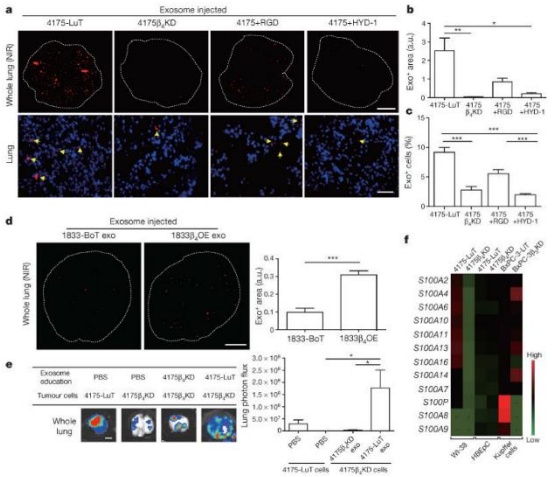
通过细胞系筛选到潜在标志物，利用临床标本进行验证，在小鼠体内进行机制研究

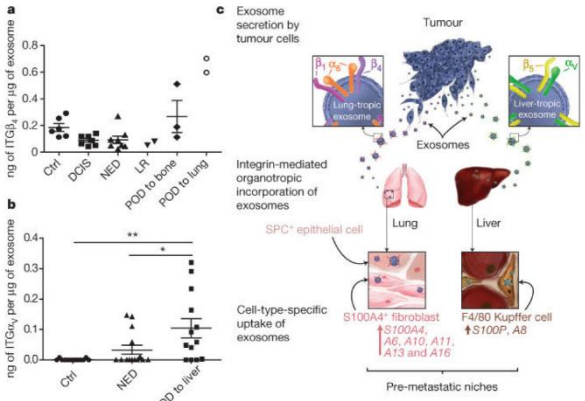
观察到不同转移倾向细胞的外泌体靶向不同器官，质谱分析发现差异蛋白整合素

敲低外泌体整合素，注射小鼠，观察细胞定植转移改变

用外泌体整合素亚型预测乳腺癌患者发生转移的器官







EVs诊断标志物研究应用的主要问题

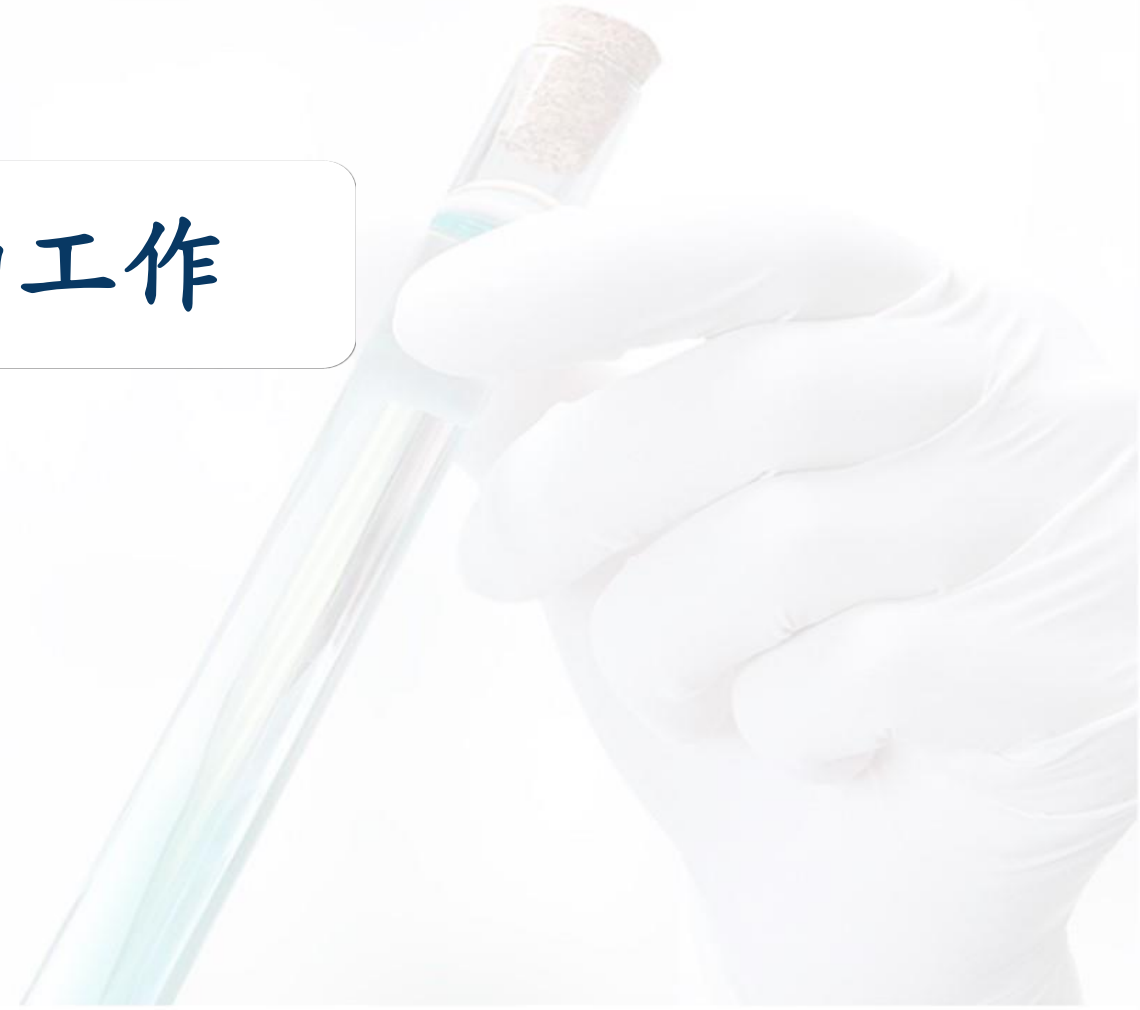
1. 快速简便的提纯方法；

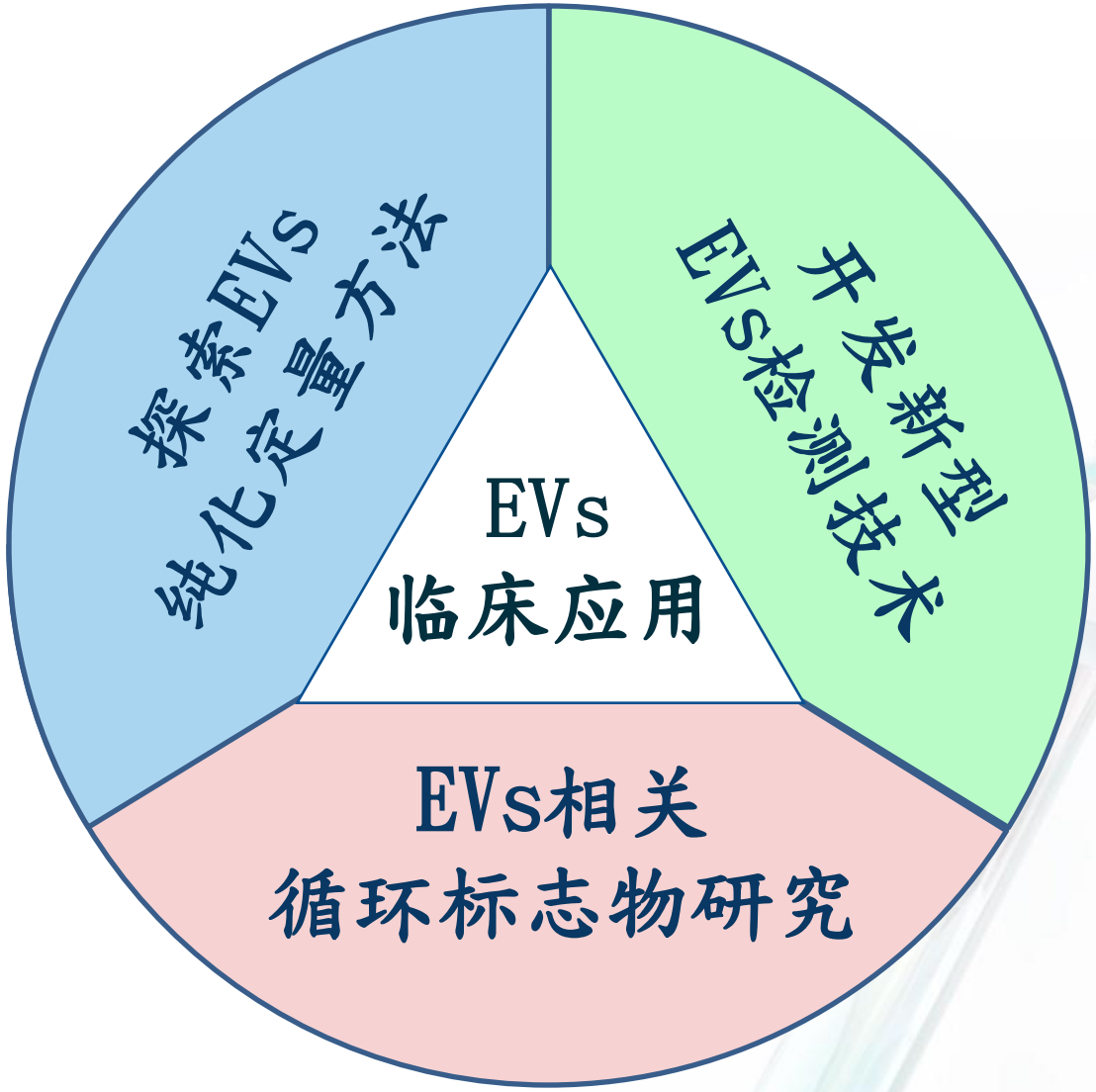
2. 鉴定器官或疾病特异性的EVs；

3. 区分EVs功能亚群；

4. 检测方法标准化。

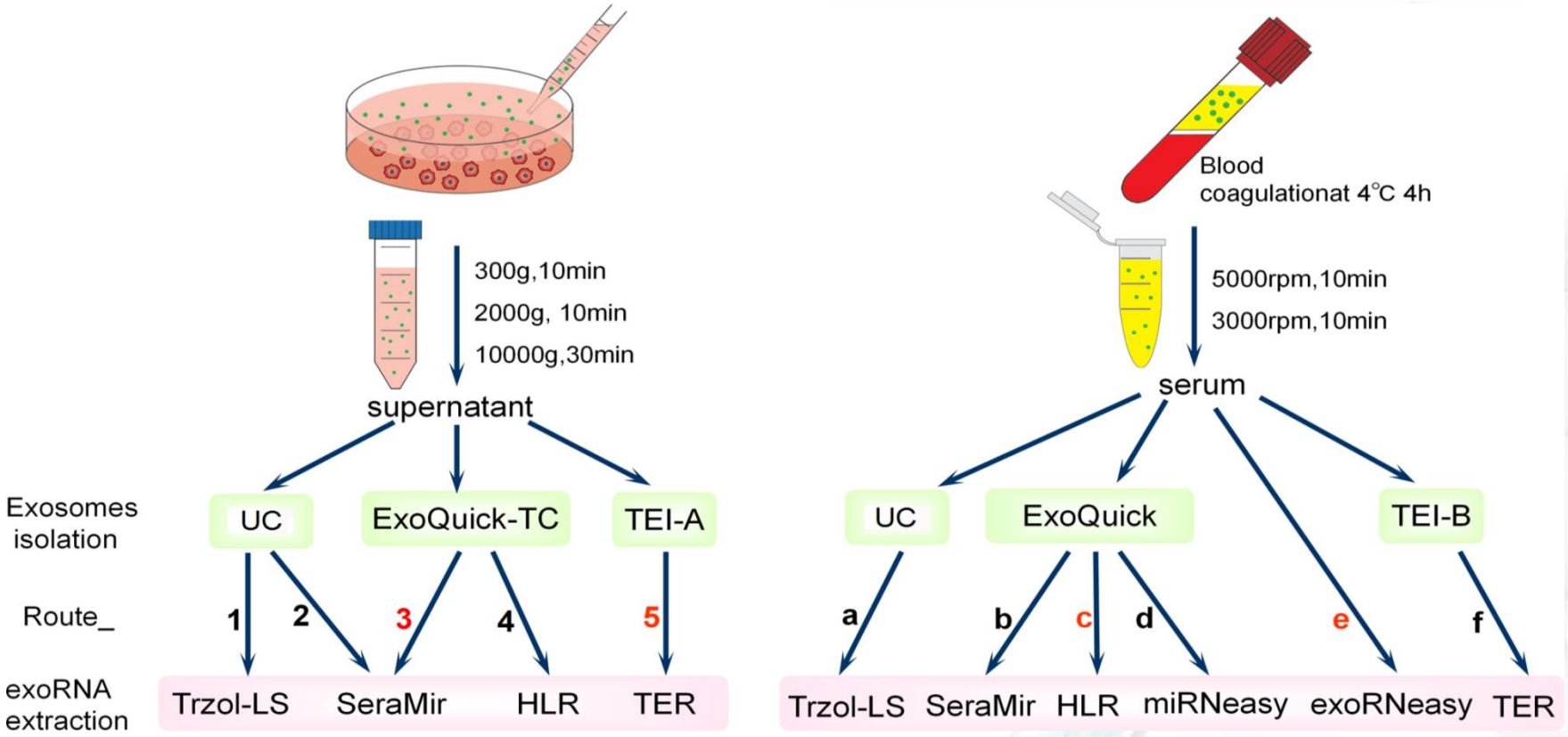
3. 我们的工作




探索EVs
纯化定量方法
开发新型
EVs检测技术
EVs
临床应用
EVs相关
循环标志物研究

多种EVs纯化定量方法比较

我们比较了多种Exosome纯化及核酸定量方法，总结出
各方法的优缺点，便于后续选择合适的EVs纯化方案



INT J MOL MED 2017, 40(3):834-844.

多种EVs纯化定量方法比较-细胞培养液

细胞培养液中Exosomes和Exosomes RNA提取方法比较

方法	提取效率				优缺点	推荐用途
	exosomes		Exo-RNA			
	浓度	纯度	浓度	纯度		
Ultra+ Trizol/Seramir	L	H	L	M	exosomes蛋白污染少，提取效率低	起始体积较大样本（CCM、尿液等），蛋白组学研究
Exoquik+ Seramir	H	L	H	L	exosomes提取效率高；有蛋白污染；exoRNA含长链RNA片段	总exoRNA分析
exoquik+HLR TEI+TER	**H**	**L**	**H**	**H**	**exosomes提取效率高；有蛋白污染；小RNA质量高，无长链干扰**	**高通量测序或其它小RNA分析研究**

注：H：高； M：中，L：低；粗体标记方法为推荐方法

INT J MOL MED 2017, 40(3):834-844.

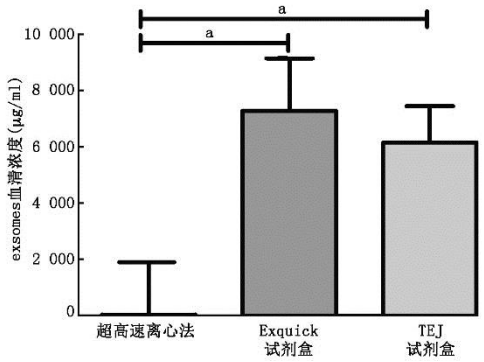
多种EVs纯化定量方法比较-血清

中华检验医学杂志 2016 年 6 月第 39 卷第 6 期 Chin J Lab Med, June 2016, Vol. 39, No. 6

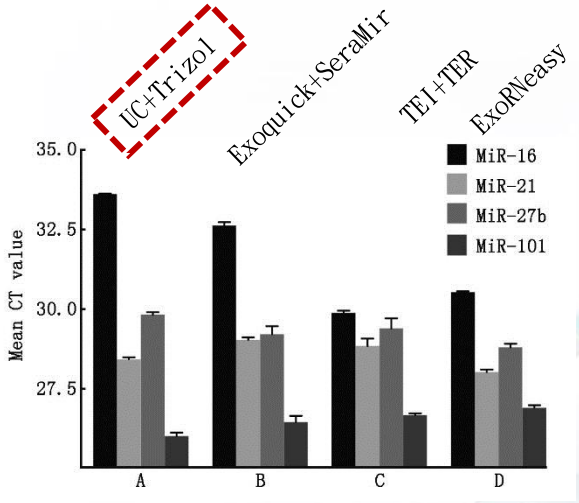
·论著·

血清中外泌体及外泌体 RNA 提取方法比较研究

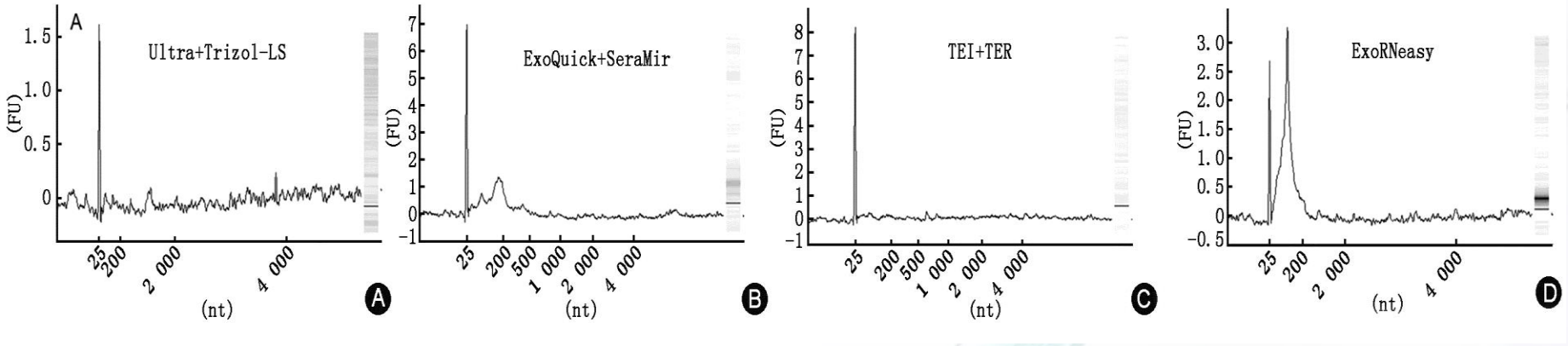
黄依瑶 唐月汀 覃思华 徐咏 安泰学 郑磊



Bradford法定量exosomes蛋白浓度



实时荧光定量PCR分析不同方法提取exo-RNA中4种miRNA表达水平

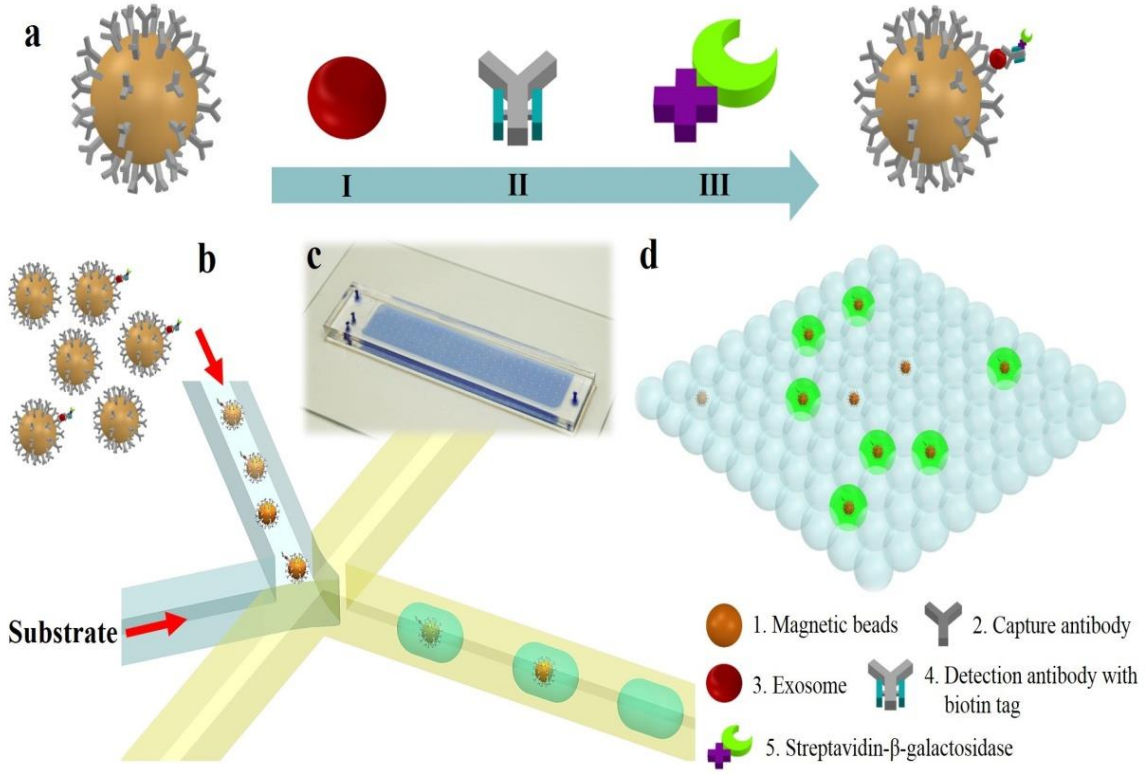


4种方法提取RNA质量分析

中华检验医学杂志，2016，39（6）

新型EVs检测技术的开发

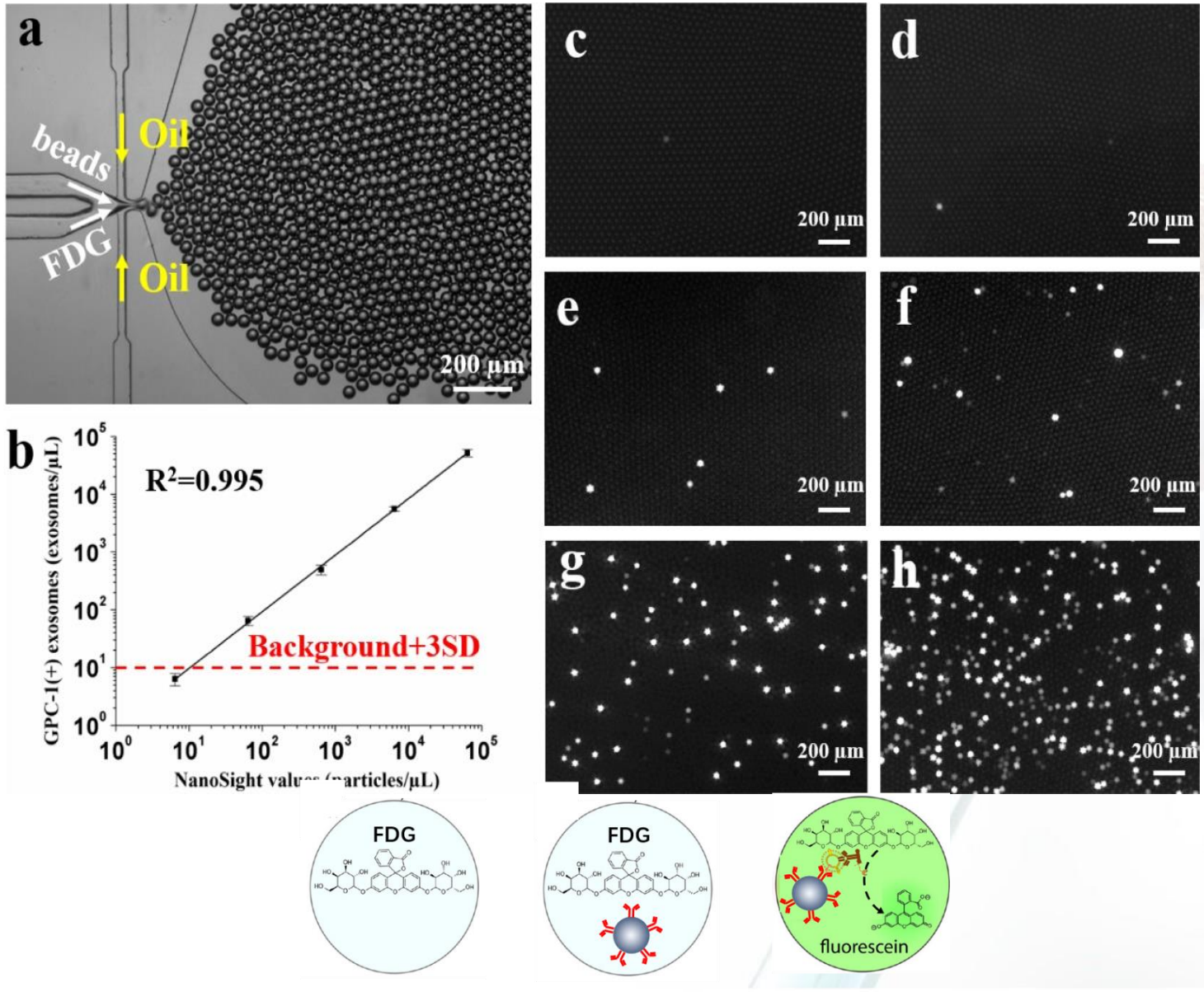
基于微液滴技术实现外泌体的单个分析及绝对定量



Shuhuai Yao, **Lei Zheng**, Xiaonan Xu, Chunchen Liu, Yu Hu, Quantification, Isolation, and Characterization of Exosomes using Droplet-based and Well-based Microfluidic Systems, 2017/10/05, USA, (62/606,687)

Nano Letters, 2018

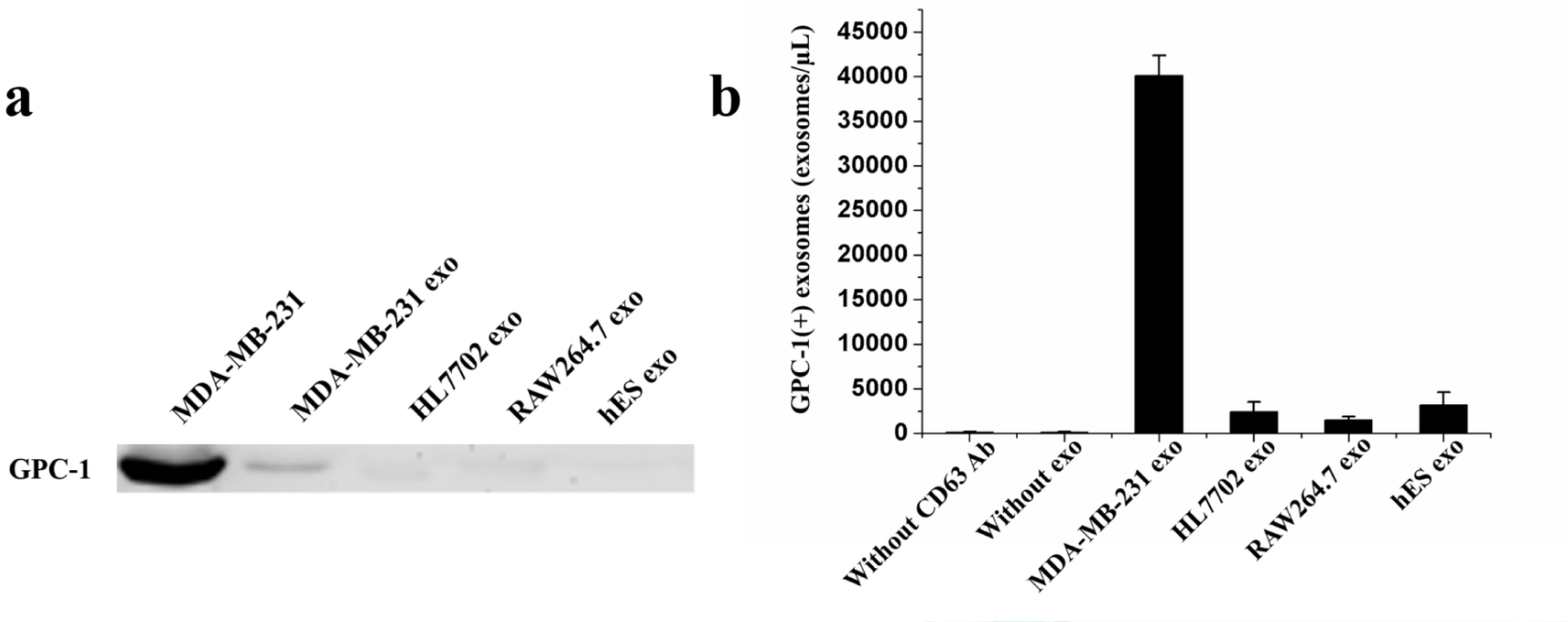
Our droplet digital ExoELISA approach is able to detect as few as ~10 exosomes/μL.



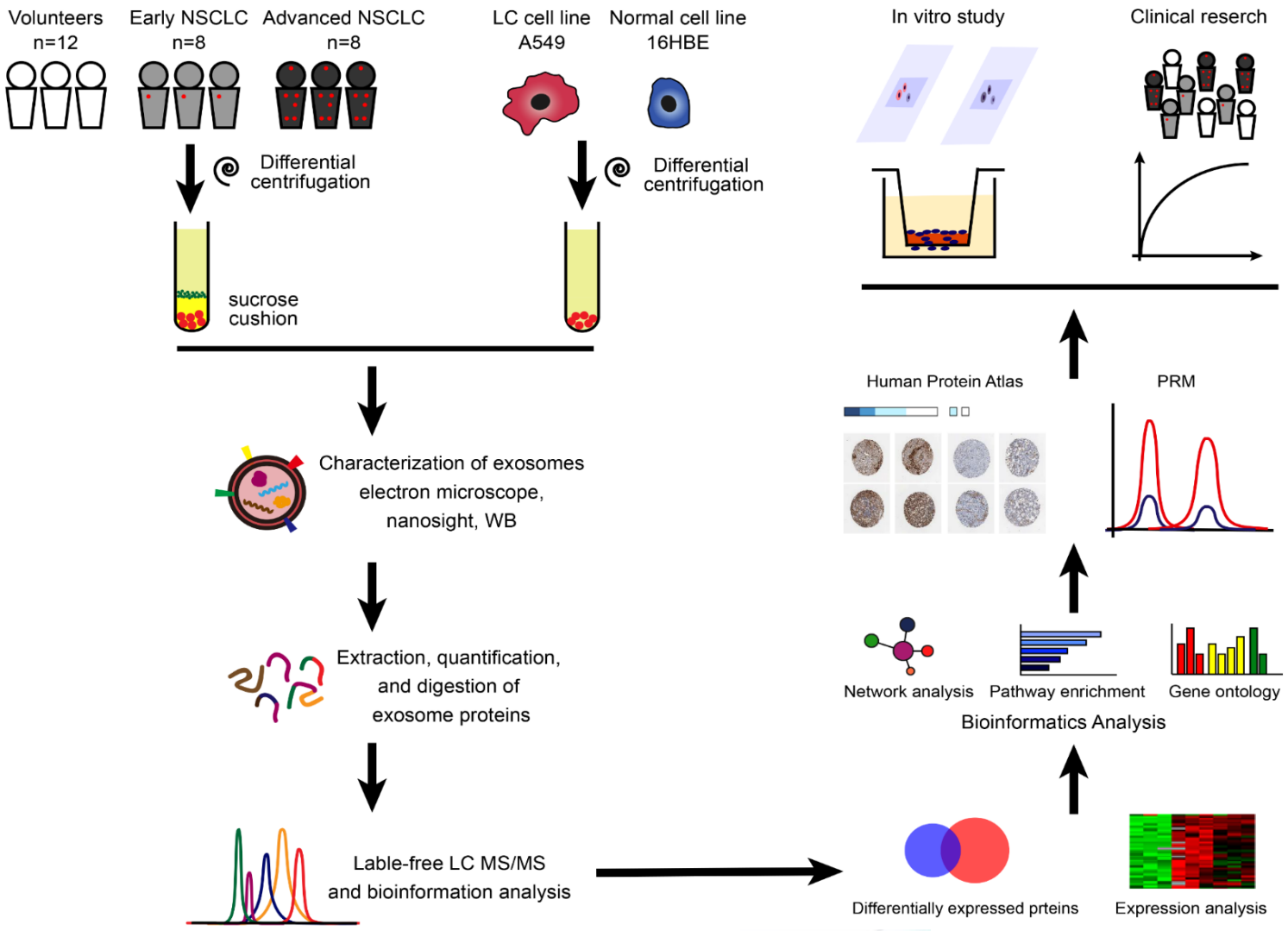
Specificity assays

two negative controls:
a sample using magnetic beads without CD63 Ab,
a sample using CD63-functionalised magnetic beads with no exosomes;

three kinds of non-cancerous exosomes:
human normal liver exosomes (HL-7702 exo),
mouse normal macrophage exosomes (RAW264.7 exo),
human embryonic stem exosomes (hES exo).



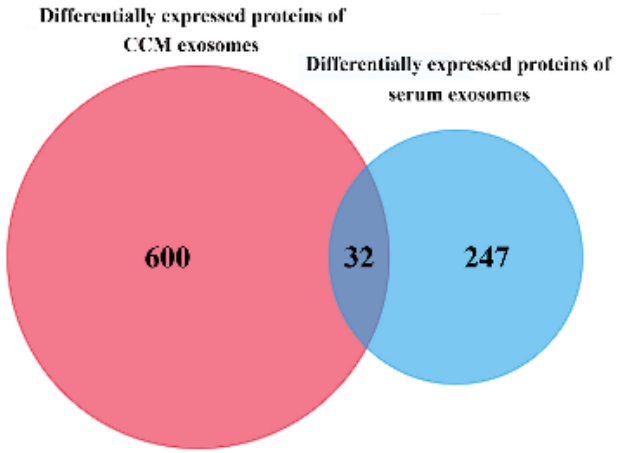
血清EVs蛋白质组学筛选肺癌生物标志物



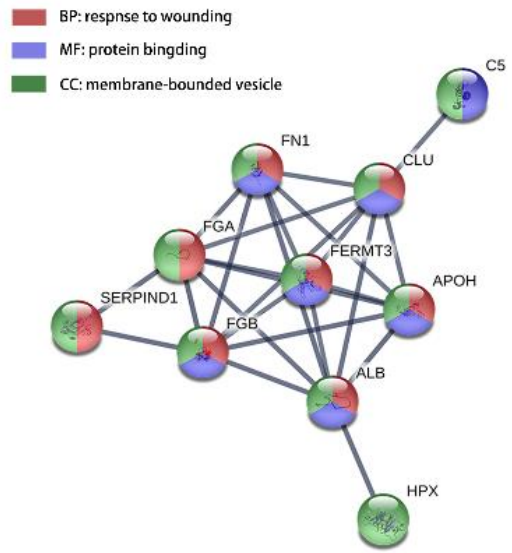
Proteomics，2019

血清EVs蛋白质组学筛选肺癌生物标志物

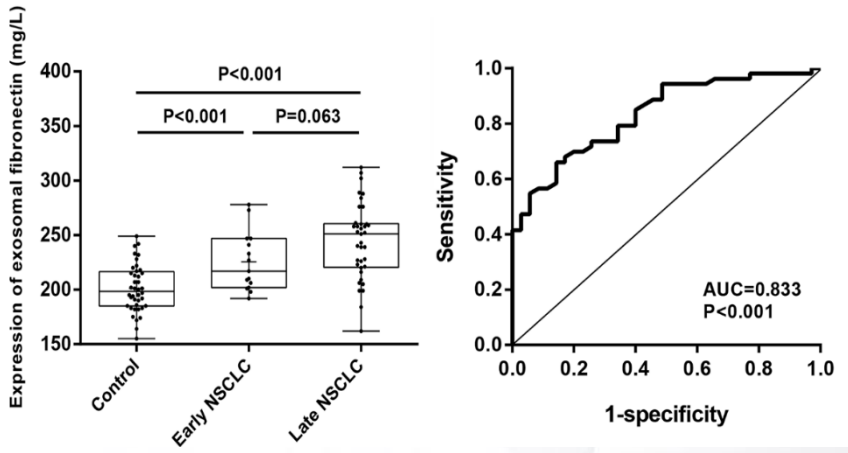
A



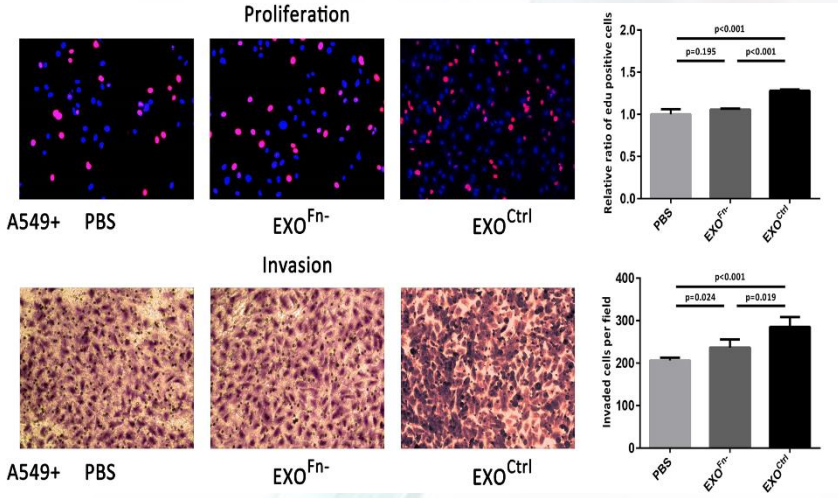
B



C

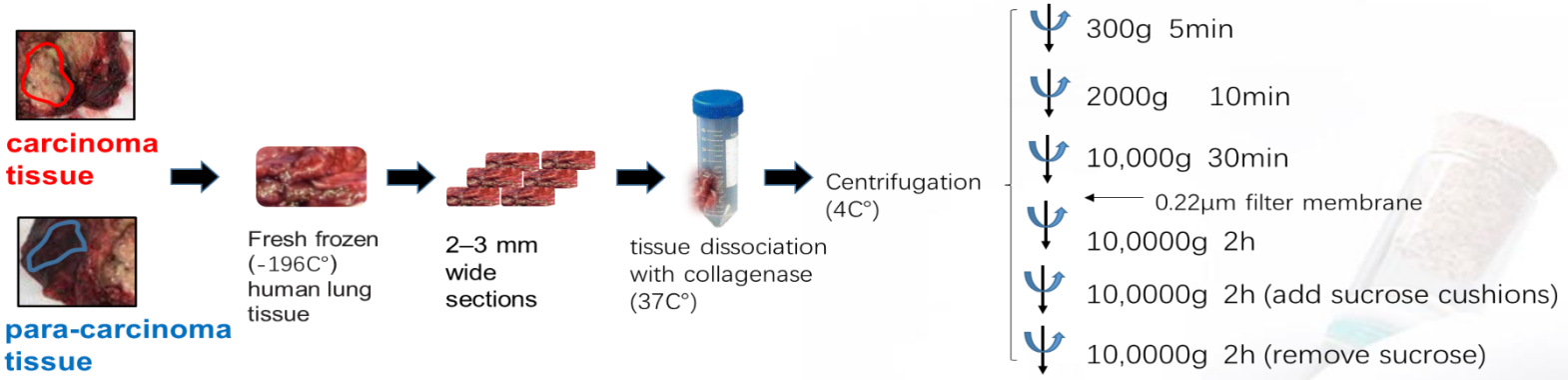


D



Proteomics，2019

基于肿瘤组织EVs蛋白质组筛选生物标志物



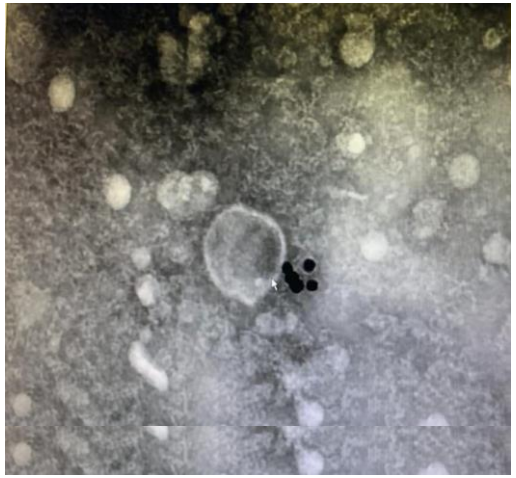
Comparisons	Significantly changing in abundance	
	Increased	Decreased
CA_vs_H	86	95

Differentially expressed EVs proteins between lung carcinoma tissue and para-carcinoma tissue and their bioinformatics analysis

Unpublished data

基于肿瘤组织EVs蛋白质组筛选生物标志物

A



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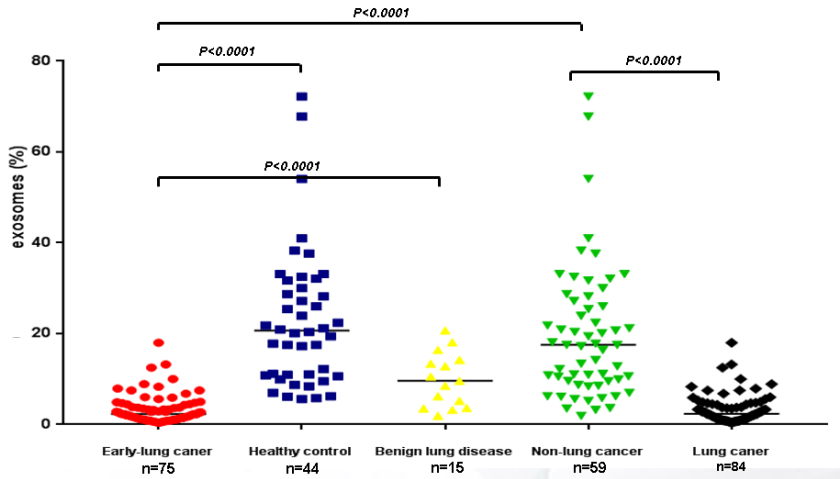
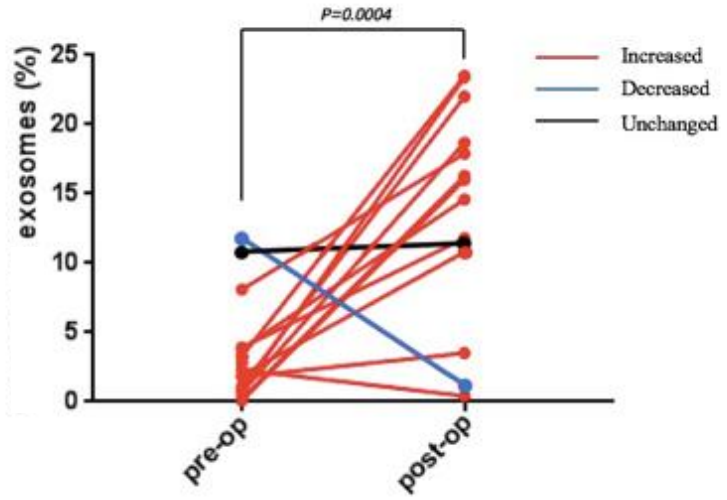
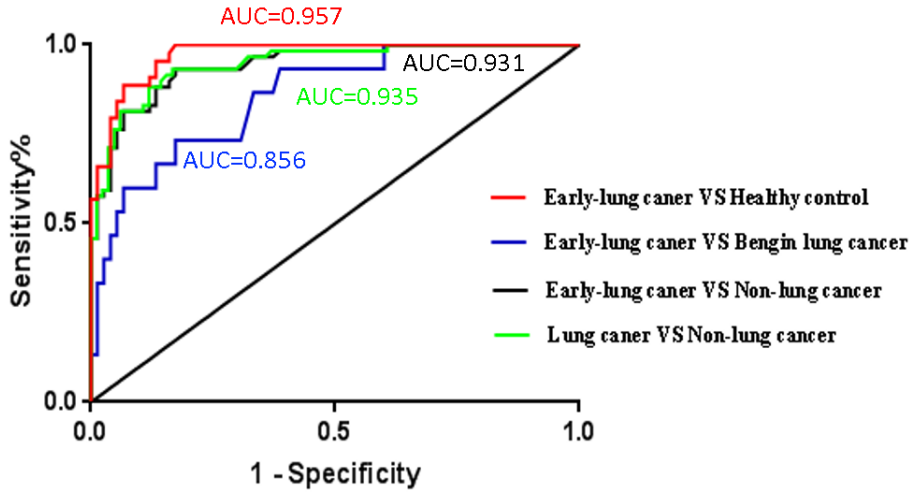


B



D



Unpublished data

4. EVs学术组织

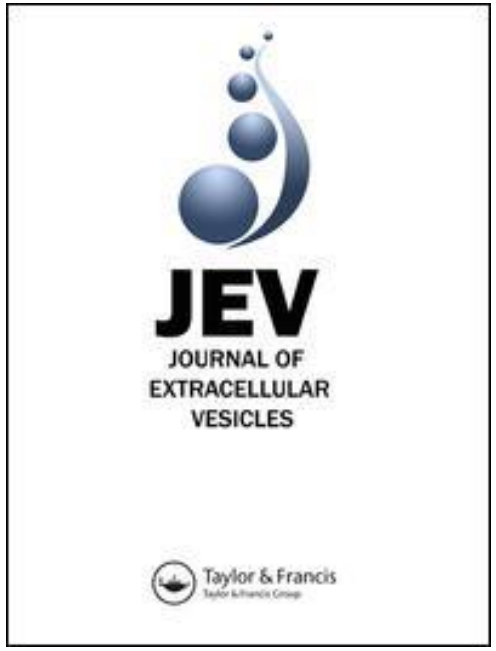
国际细胞外囊泡协会（ISEV）

2011年国际囊泡协会（International Society For Extracellular Vesicles，ISEV）

创办官方会刊 Journal of Extracellular Vesicles（JEV）

召开年会时间	地点	参会人数
2012，April 18-20	Gothenburg, The Sweden	480
2013，April 17-20	Boston,The USA	
2014，April 30 - May3	Rotterdam,The Netherlands	685
2015，April 23-26	Washington DC,The USA	780
2016，May 4-7	Rotterdam, The Netherlands	860
2017，May 18-21	Toronto, Canada	960
2018， May 2-6	Barcelona, Spain	1200
2019， April 24-28	Kyoto, Japan	1200

Journal of extracellular vesicles (JEV)



- Online ISSN: 2001-3078；
- 国际囊泡协会ISEV会刊；
- 严格同行评议审查，免费获得全文；
- 文章形式：original research, review, case report, meeting report, technical report；
- 版面费：ISEV会员，7页以内文章€950/篇；
- 年发文章量：约60篇；
- 2018年影响因子: 11

Editors-in-Chief

Clotilde Thery, Institut Curie, INSERM, France

Peter J. Quesenberry, Brown University, United States

Yong Song Gho, Pohang University of Science and Technology, Korea

ISEV 走进亚洲

2018 ISEV Guangzhou Workshop

EV-based Clinical Theronositics

2018.11.18-20, Guangzhou, Southern Medical University

CSEV 2017年成立

2017年6月 中国广州

学会官网：www.nflab.net/CSEV/

2018 2nd CSEV Annual Meeting

Venue: Guangzhou, Guangdong Province

Time: 2018.11.16-18

2019 3rd CSEV Annual Meeting

2019 第三届全国细胞外囊泡大会

时间： 2019年10月18日-20日

ISEV 2020 Annual Meeting

ANNUAL MEETING ISEV 2020
20-24 May
Philadelphia Marriott Downtown
Philadelphia, Pennsylvania

The ISEV2020 Annual Meeting will be held 20-24 May
at the Marriott Philadelphia Downtown Hotel.

Export to Your Calendar 5/20/2020 to 5/24/2020
When: Wednesday, May 20, 2020
Where: Philadelphia Marriott Downtown
1201 Market St Philadelphia, Pennsylvania 19107, United States
Contact: contact@isev.org


Thanks!