Adviser: Dr. Hajar Mardani

The accumulation of exosome-associated microRNA-1246 and microRNA150-3p in human red blood cell suspensions

Presented by: Mahdiyeh Abbaspoor

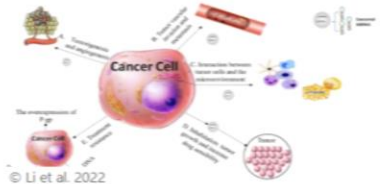
Journal of Translational Medicine

Featured Section: Disease Biomarkers

The Disease Biomarkers section aims to bridge the identification and validation of discovered gene or protein-based, network or dynamic network biomarkers with human diseases, patient phenotypes, or clinical applications, and accelerate the development of human disease-specific biomarkers for the early diagnosis, monitoring, evaluation, and prediction of diseases.

Edited by Xiangdong Wang, the section welcomes research on the development of advanced biotechnologies for biomarker discovery and development, identifying biomarkers associated with the early detection of diseases, monitoring of disease severity and duration as well as patient responses to therapies, prediction of patient outcomes and the evaluation, regulation and ethics of disease biomarkers.

Read the latest articles in the *Disease Biomarkers* section



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Speed
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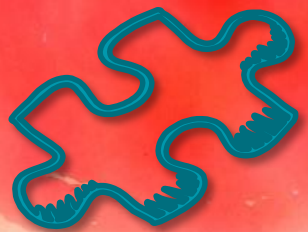
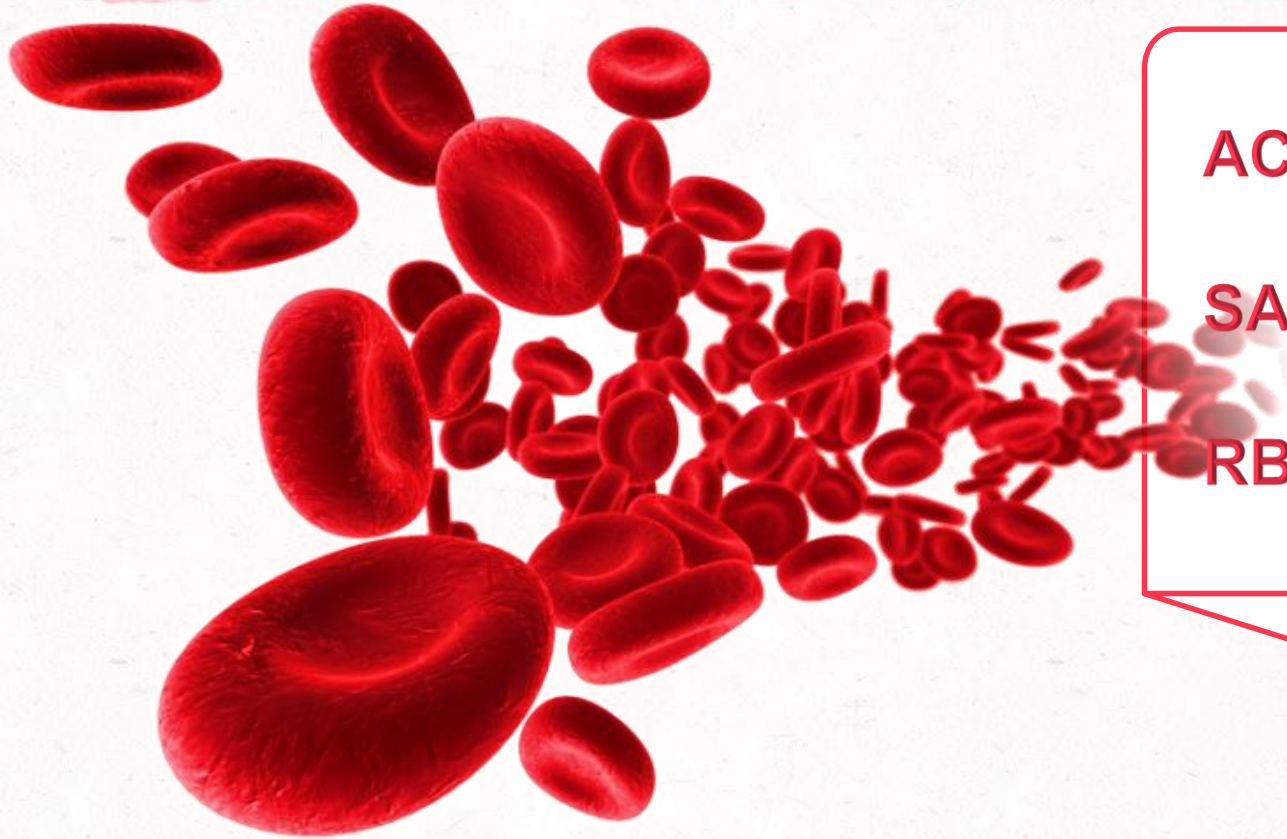
5.531 - 2-year Impact Factor
5.760 - 5-year Impact Factor
1.244 - Source Normalized

1. Background



Red blood cell suspension



ACD-A 35 days at 4

SAG-M 42 days

RBC storage lesions

Complications and Adverse Clinical Outcomes

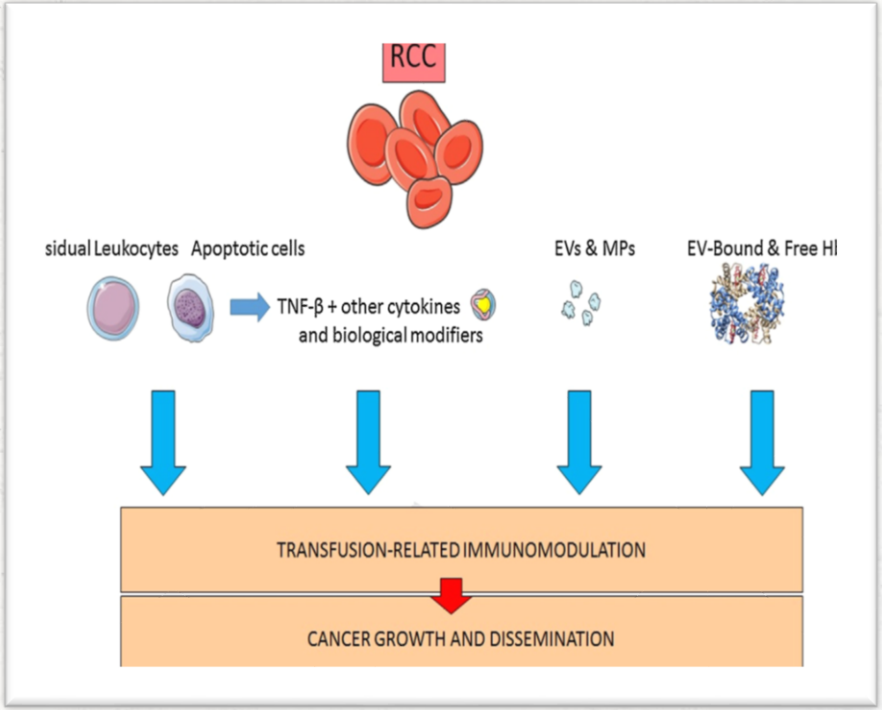
Cardiovascular → Thrombotic Complications.

Trauma → Deep Vein Thrombosis and Bacterial Infections

Transfusion-related immunomodulation

TRIM

Progression and Prognosis of an Existing Disease



KIDNEY TRANSPLANTATION

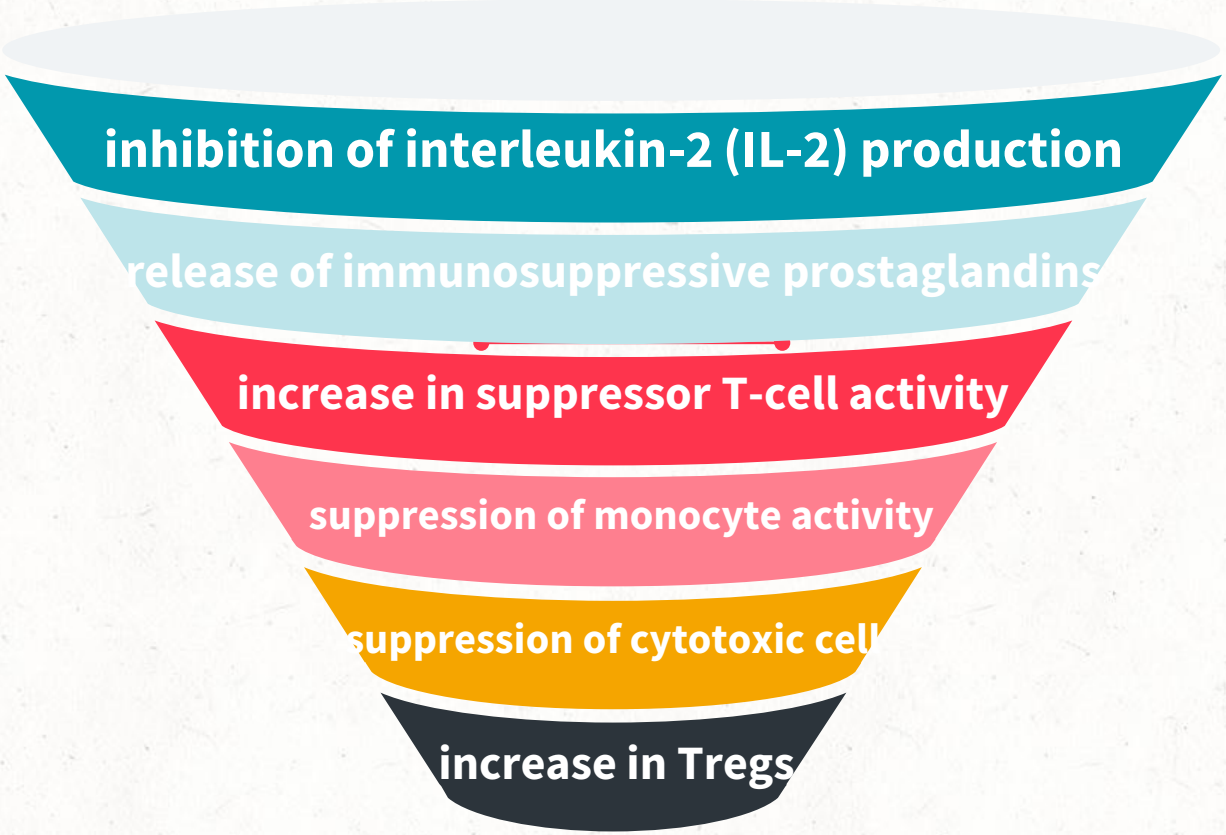
Abortion

Tumor incidence

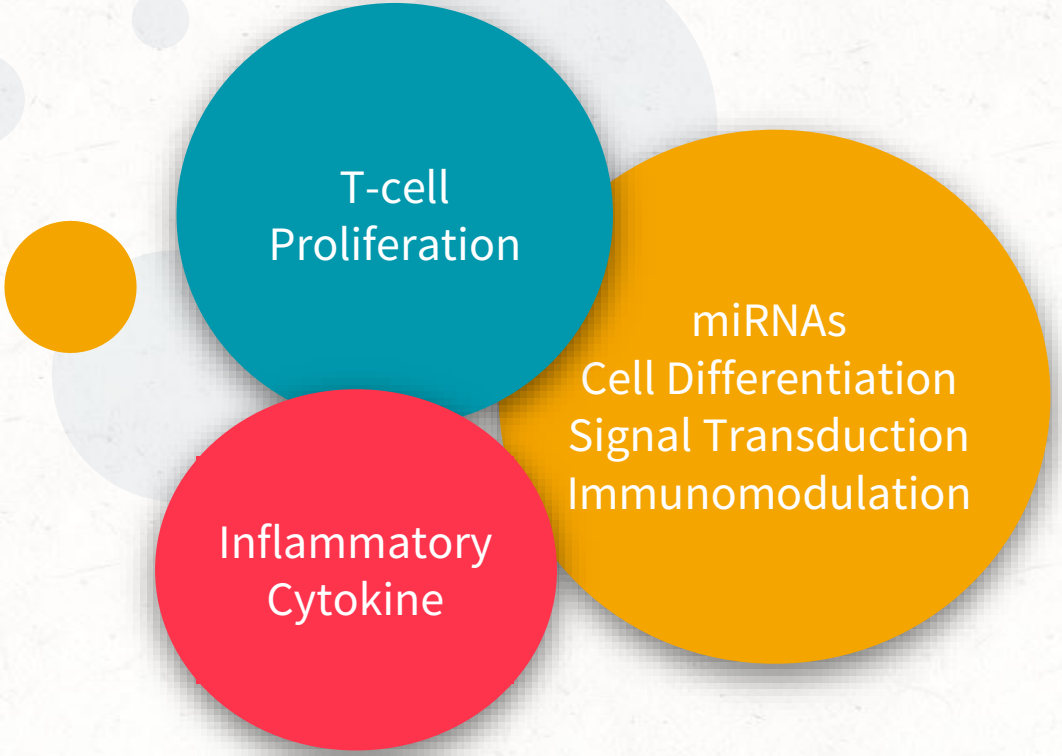
pathogen infection

Mortality

Mechanisms


inhibition of interleukin-2 (IL-2) production
release of immunosuppressive prostaglandins
increase in suppressor T-cell activity
suppression of monocyte activity
suppression of cytotoxic cell
increase in Tregs

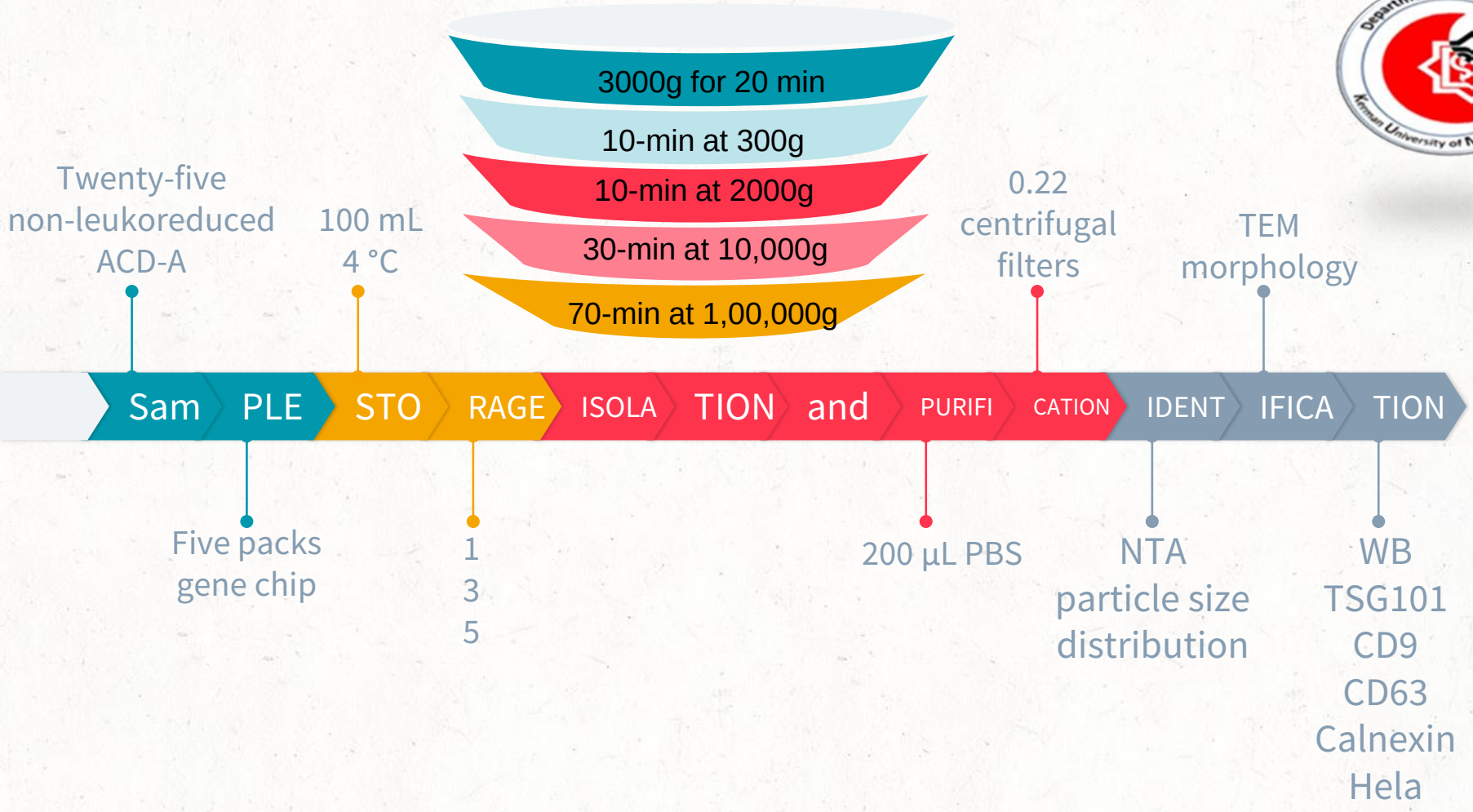
Exosomes


T-cell
Proliferation
miRNAs
Cell Differentiation
Signal Transduction
Immunomodulation
Inflammatory
Cytokine

This study

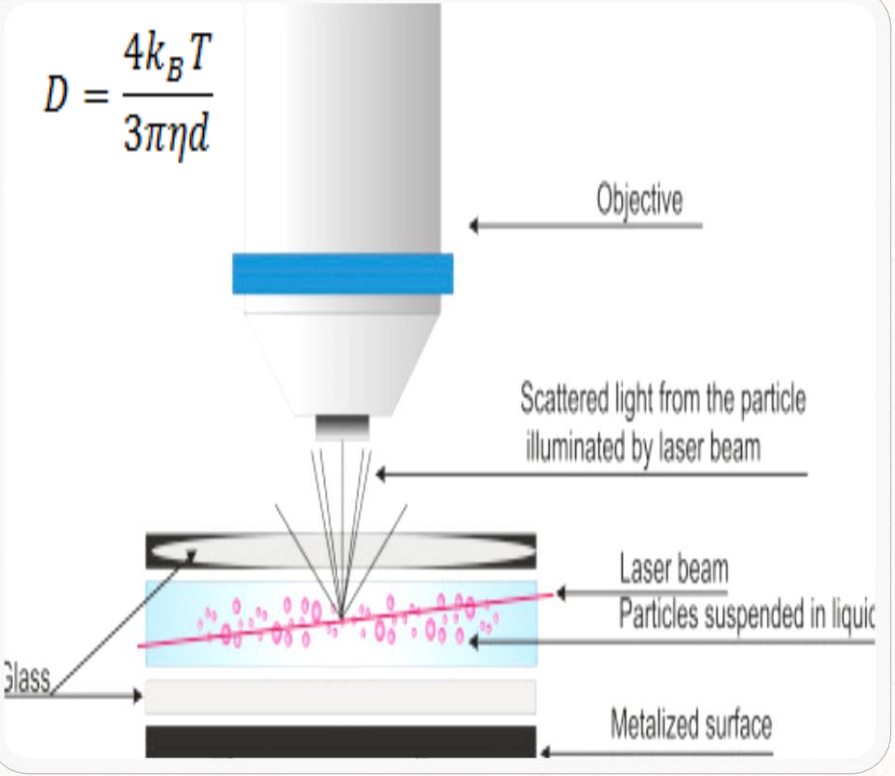

miRNA Contained in Exosomes
Dynamic Alterations in the Expression of Exosomal miRNA
Potential Functions Associated with Immunoregulation

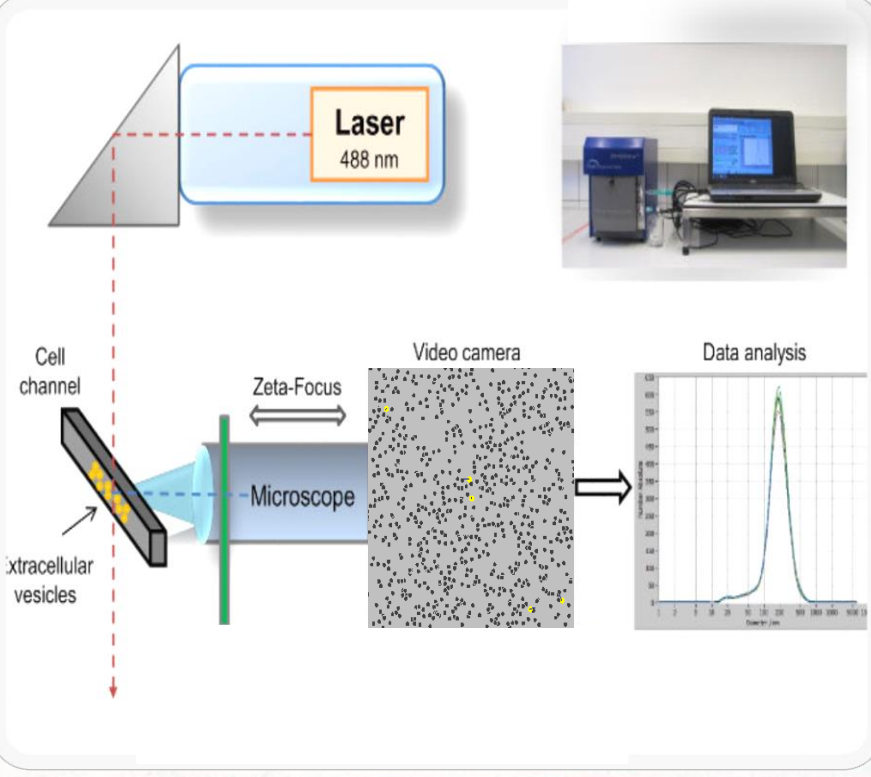
2. Materials and Methods

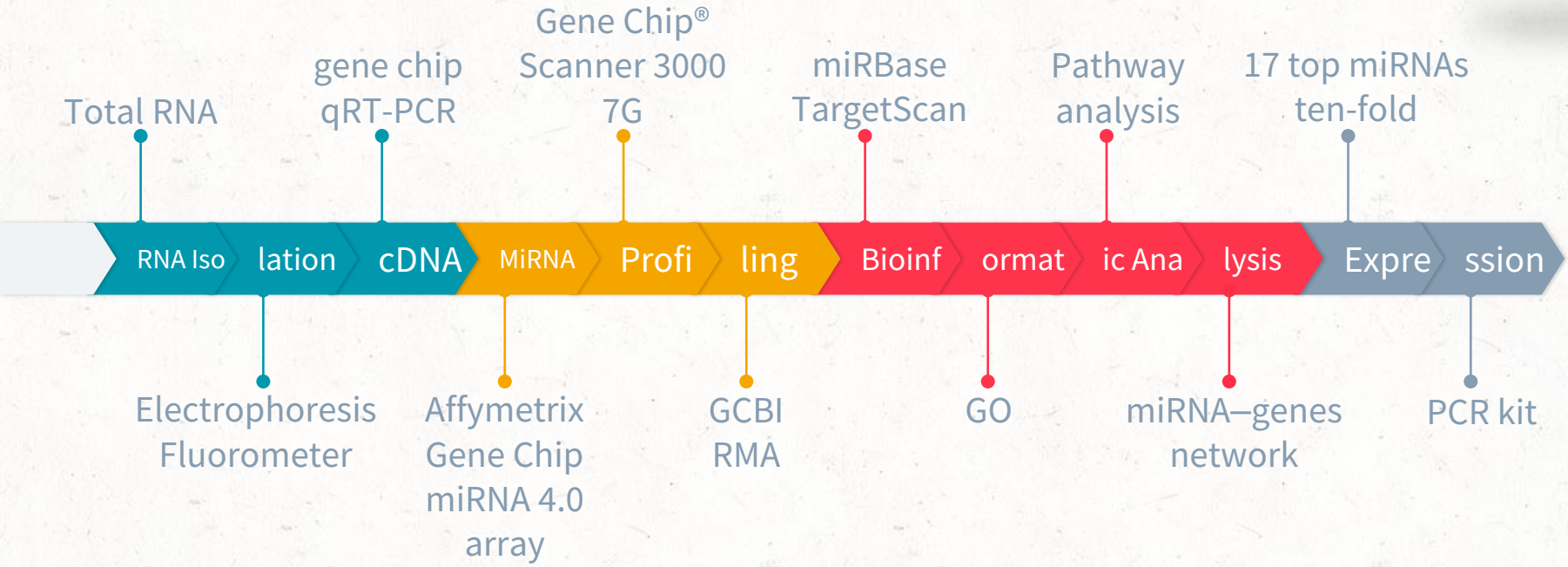

3000g for 20 min
10-min at 300g
10-min at 2000g
30-min at 10,000g
70-min at 1,00,000g
Twenty-five non-leukoreduced ACD-A
100 mL 4 °C
0.22 centrifugal filters
TEM morphology
Sam PLE STO RAGE ISOLA TION and PURIFI CATION IDENT IFICA TION
Five packs gene chip
1 3 5
200 µL PBS
NTA particle size distribution
WB TSG101 CD9 CD63 Calnexin Hela

NANOPARTICLE TRACKING ANALYSIS

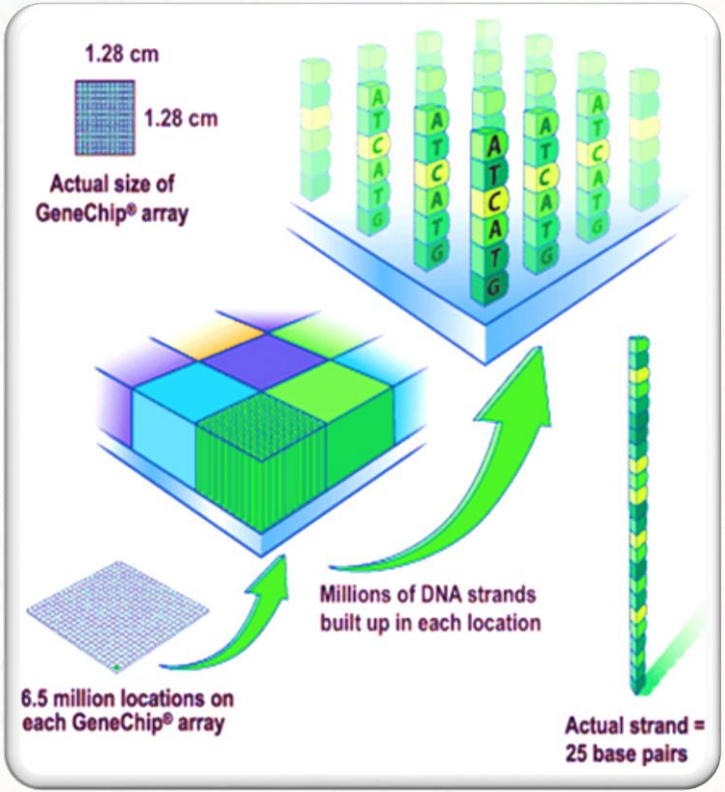
$$D = \frac{4k_B T}{3\pi\eta d}$$

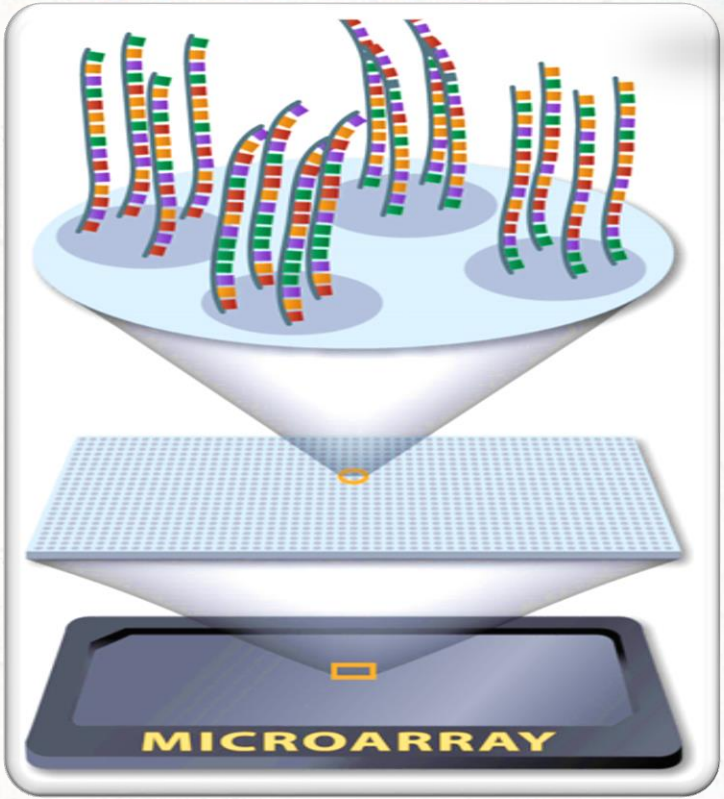


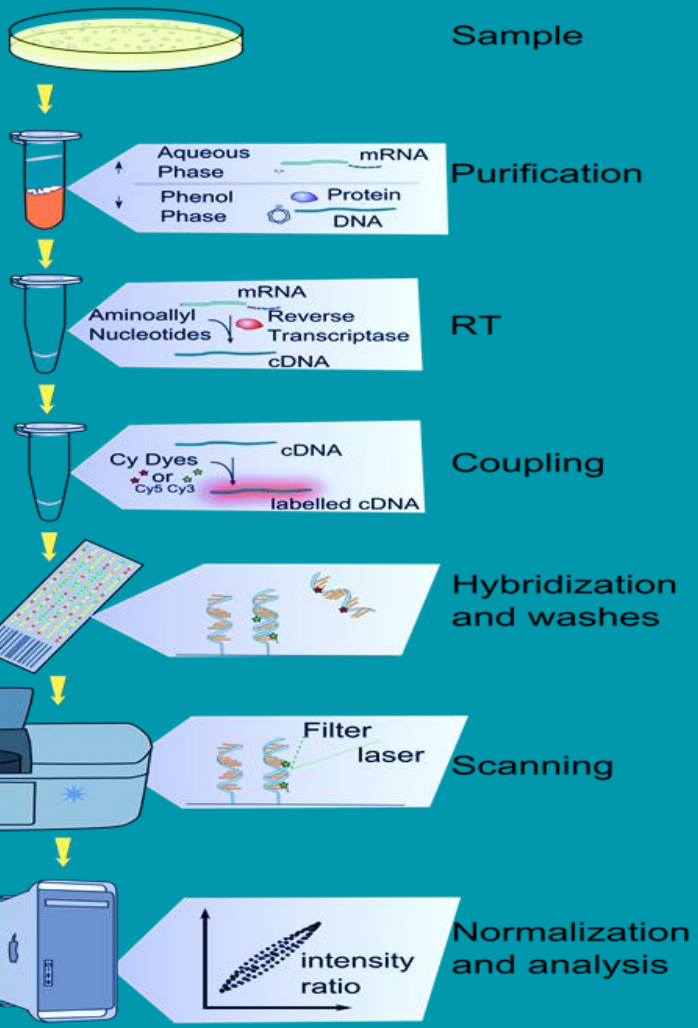


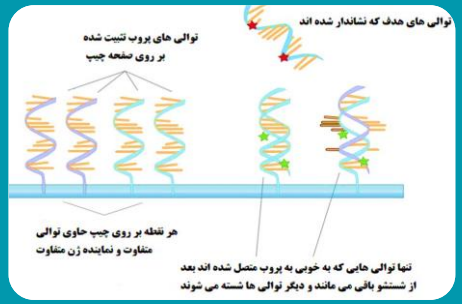

Total RNA
gene chip
qRT-PCR
Gene Chip®
Scanner 3000
7G
miRBase
TargetScan
Pathway
analysis
17 top miRNAs
ten-fold
RNA Iso
lation
cDNA
MiRNA
Profi
ling
Bioinf
ormat
ic Ana
lysis
Expre
ssion
Electrophoresis
Fluorometer
Affymetrix
Gene Chip
miRNA 4.0
array
GCBI
RMA
GO
miRNA–genes
network
PCR kit

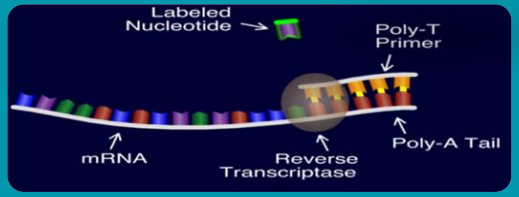
Gene Chip

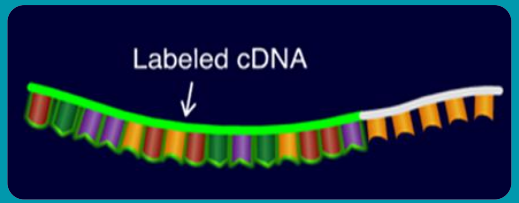

1.28 cm
1.28 cm
Actual size of GeneChip® array
Millions of DNA strands built up in each location
6.5 million locations on each GeneChip® array
Actual strand = 25 base pairs

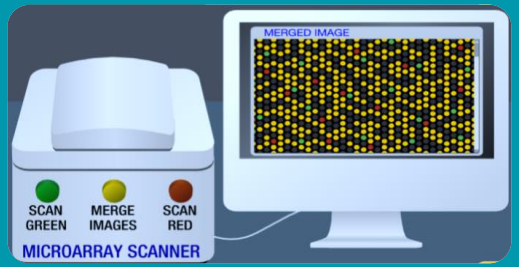

MICROARRAY


Sample
Aqueous Phase
mRNA
Phenol Phase
Protein
DNA
Purification
mRNA
Aminoallyl Nucleotides
Reverse Transcriptase
cDNA
RT
Cy Dyes or Cy5 Cy3
cDNA
labelled cDNA
Coupling
Hybridization and washes
Filter
laser
Scanning
intensity ratio
Normalization and analysis

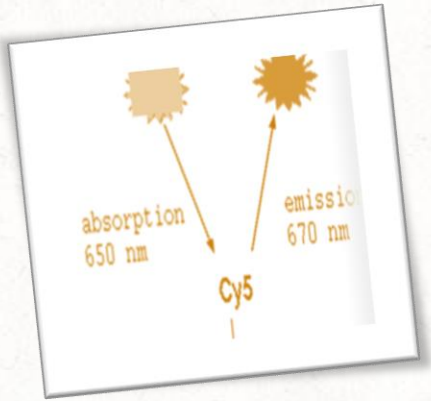

توالی های هدف که نشاندار شده اند
توالی های پروب تثبیت شده
بر روی تصفحه چیپ
هر نقطه بر روی چیپ حاوی توالی
متفاوت و نماینده ژن متفاوت
تنها توالی هایی که به خوبی به پروب متصل شده اند بعد
از شستشو باقی می مانند و دیگر توالی ها شسته می شوند

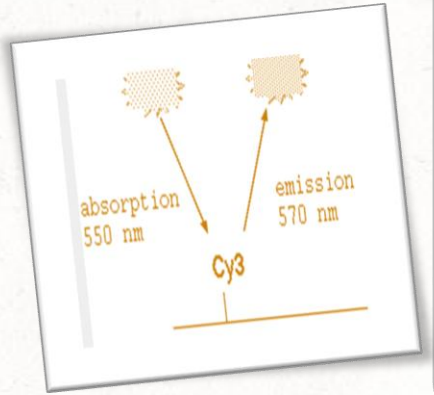

Labeled Nucleotide
Poly-T Primer
mRNA
Reverse Transcriptase
Poly-A Tail

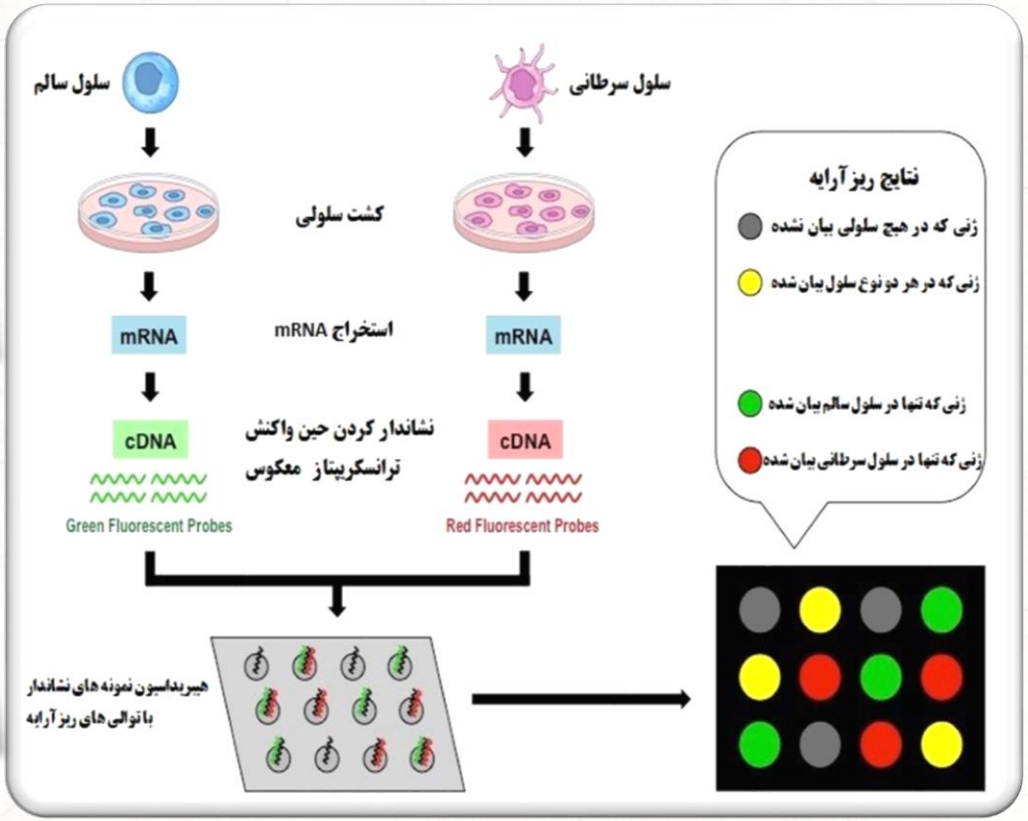

Labeled cDNA

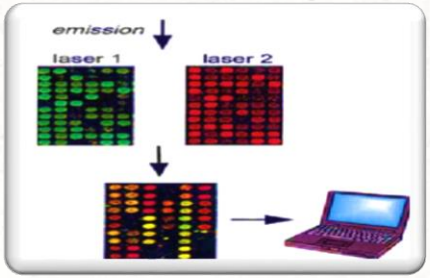

MERGED IMAGE
SCAN GREEN
MERGE IMAGES
SCAN RED
MICROARRAY SCANNER

Gene Chip


absorption
650 nm
emissio
670 nm
Cy5


absorption
550 nm
emission
570 nm
Cy3


سلول سالم
سلول سرطانی
کشت سلولی
mRNA
استخراج mRNA
mRNA
cDNA
نشاندار کردن حین واکنش
ترانسکریپتاز معکوس
cDNA
Green Fluorescent Probes
Red Fluorescent Probes
هیبریداسیون نمونه های نشاندار
با توالی های ریز آرایه
نتایج ریز آرایه
ژنی که در هیچ سلولی بیان نشده
ژنی که در هر دو نوع سلول بیان شده
ژنی که تنها در سلول سالم بیان شده
ژنی که تنها در سلول سرطانی بیان شده

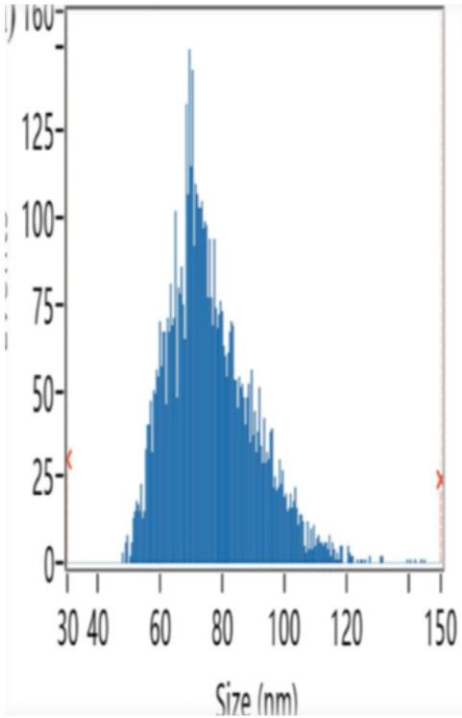

emission
laser 1
laser 2

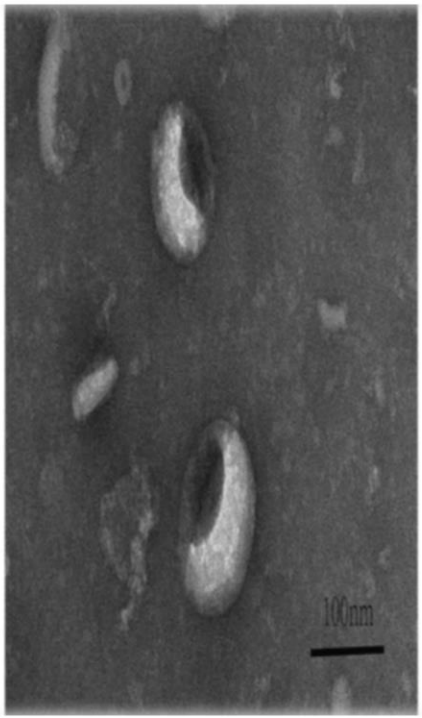
3. RESULTS

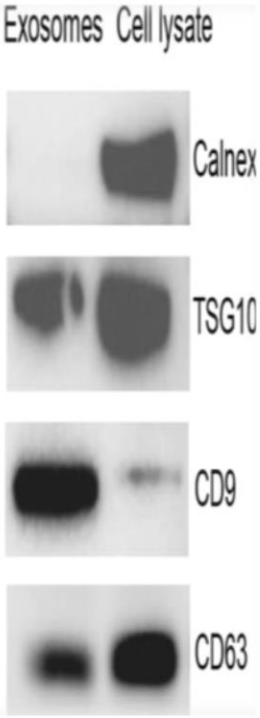
13.65 ± 76.07

95% below the 120 nm

99.98% below 150 nm







539

159 (148↑ and 11↓)

59

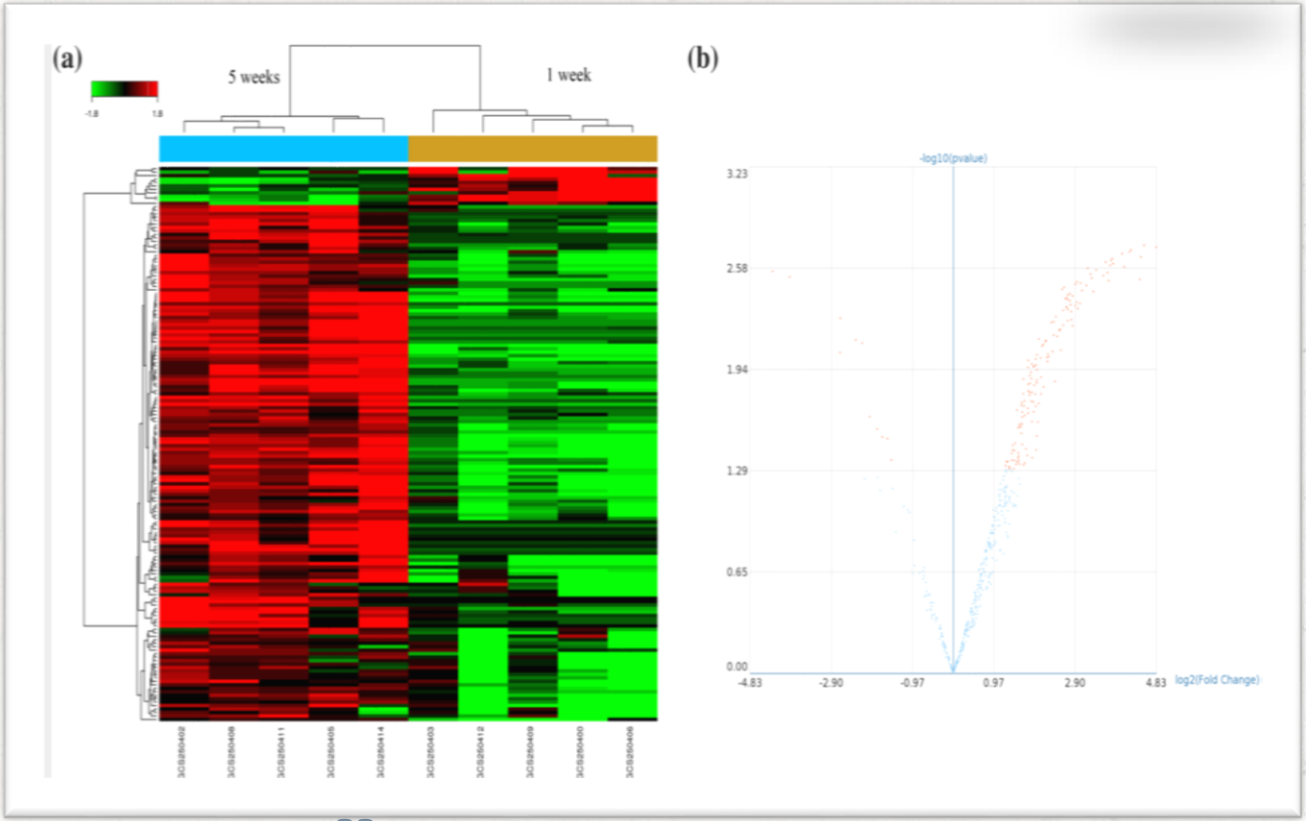
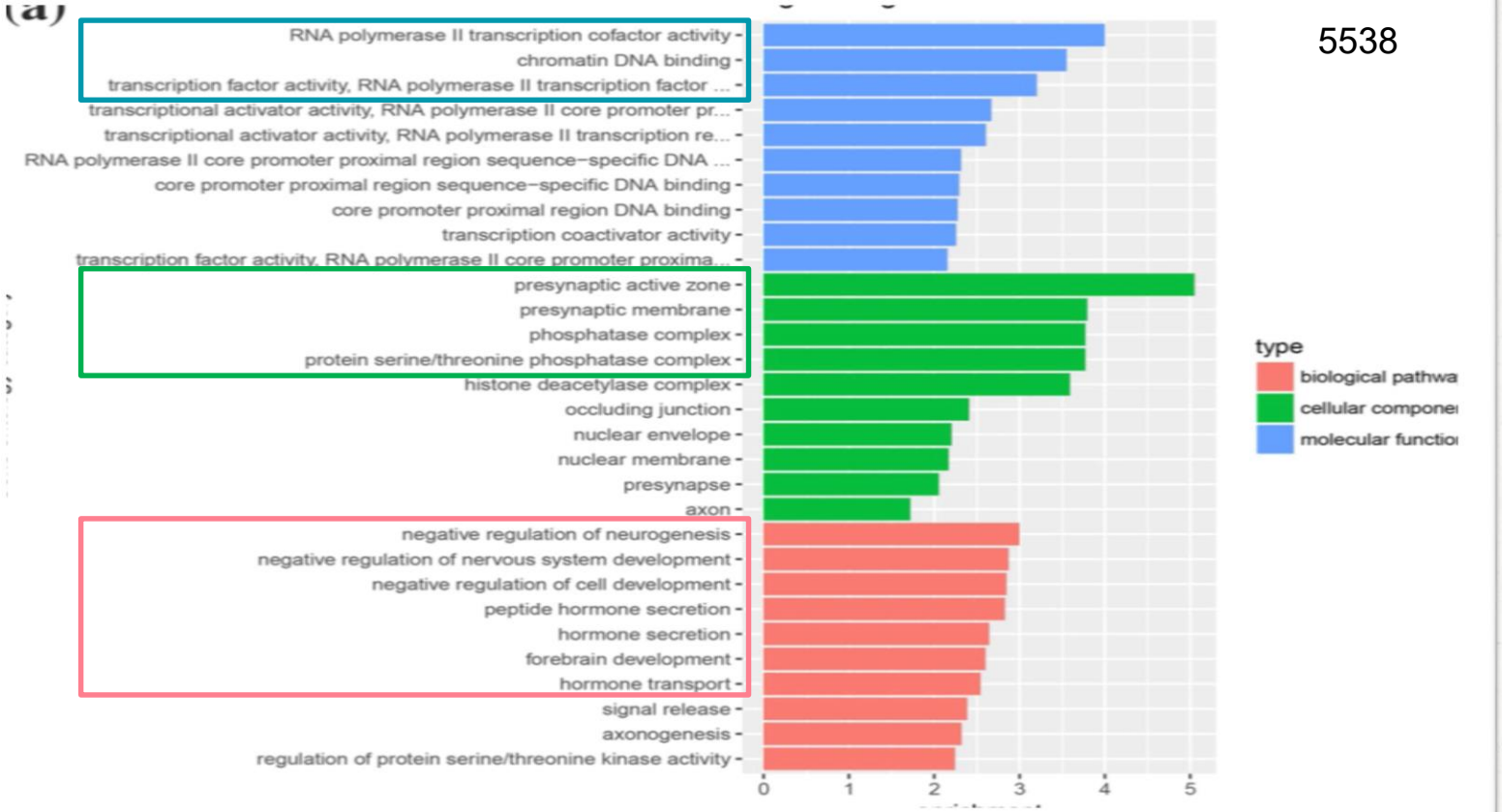
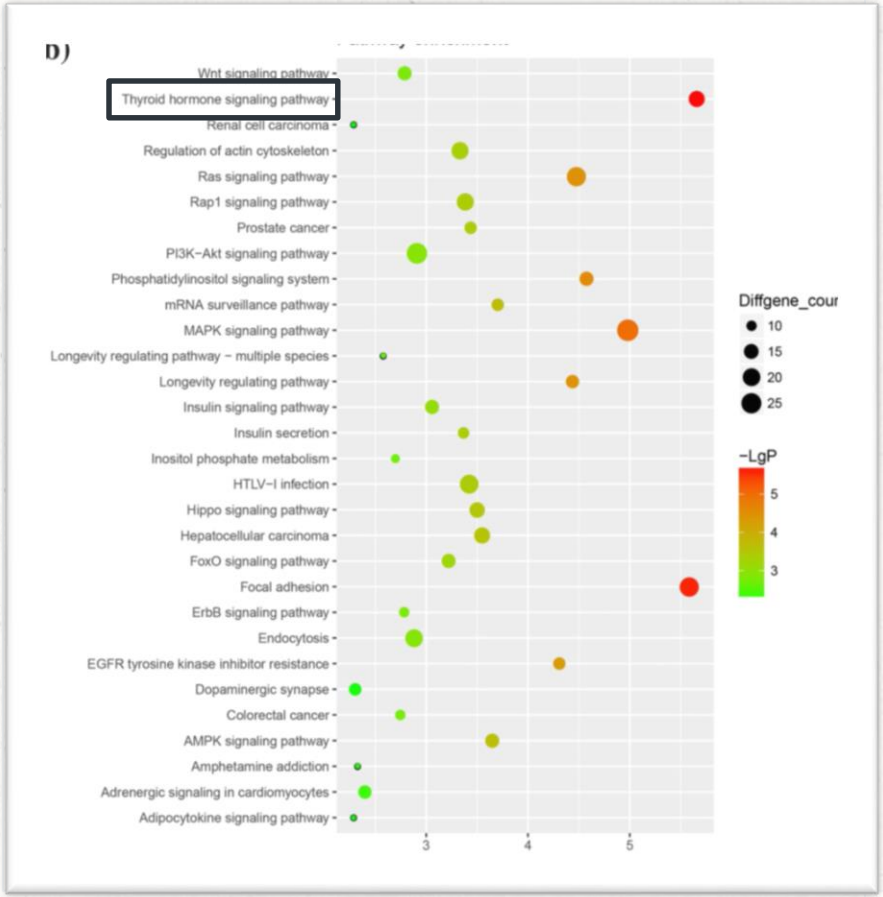


Table1 List of differential exosomal miRNAs accumulated more than tenfold at week 5 vs. week 1 storage time of RBC suspensions

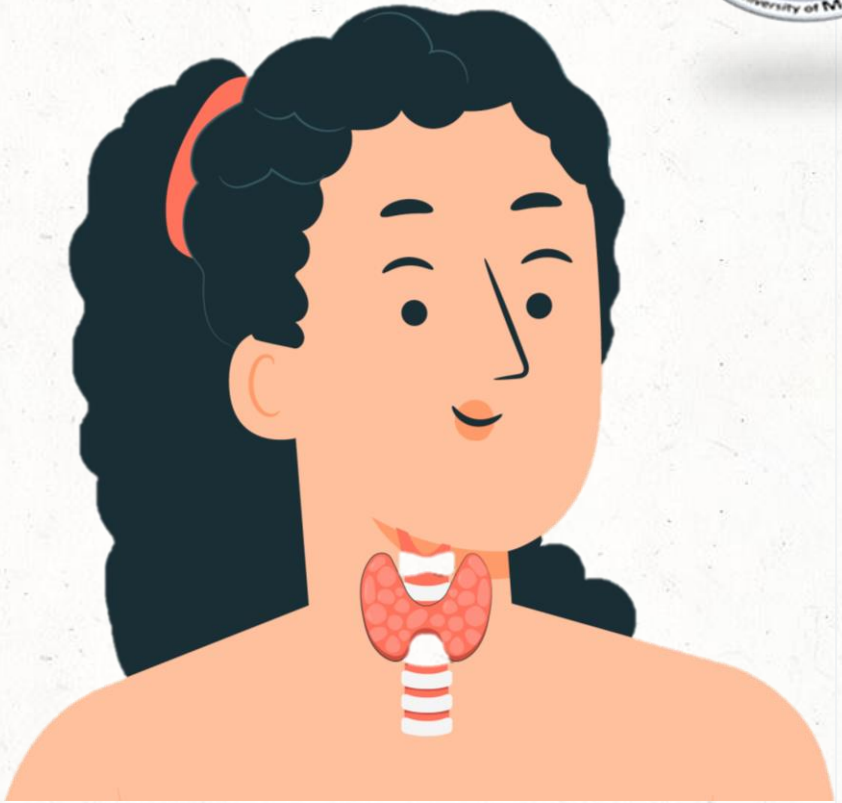
miRNA	week 5 $\log_2$ mean signal	week 1 $\log_2$ mean signal	week 5 vs. week 1 fold change	*P* value
hsa-miR-6824-5p	6.3266	1.7901	23.2080	0.0019
hsa-miR-6716-5p	7.7924	3.3319	22.0159	0.0022
hsa-miR-1246	8.4004	3.9677	21.5962	0.0031
hsa-miR-939-5p	6.9238	2.6953	18.7462	0.0020
hsa-miR-4433-3p	7.4835	3.2970	18.2076	0.0021
hsa-miR-4701-3p	6.6046	2.5412	16.7191	0.0026
hsa-miR-6849-5p	5.9334	1.9205	16.1438	0.0021
hsa-miR-595	5.7246	1.9235	13.9395	0.0024
hsa-miR-150-3p	6.0808	2.2974	13.7697	0.0023
hsa-miR-7844-5p	5.6850	1.9356	13.4488	0.0023
hsa-miR-6798-5p	8.3634	4.6340	13.2639	0.0025
hsa-miR-4689	7.9570	4.2640	12.9339	0.0031
hsa-miR-1180-3p	6.1168	2.4509	12.6924	0.0028
hsa-miR-3064-5p	5.4298	1.8060	12.3269	0.0024
hsa-miR-1225-5p	6.5773	3.0099	11.8550	0.0029
hsa-miR-6819-5p	5.6768	2.2801	10.5317	0.0027
hsa-miR-1268b	7.3709	4.0387	10.0714	0.0037

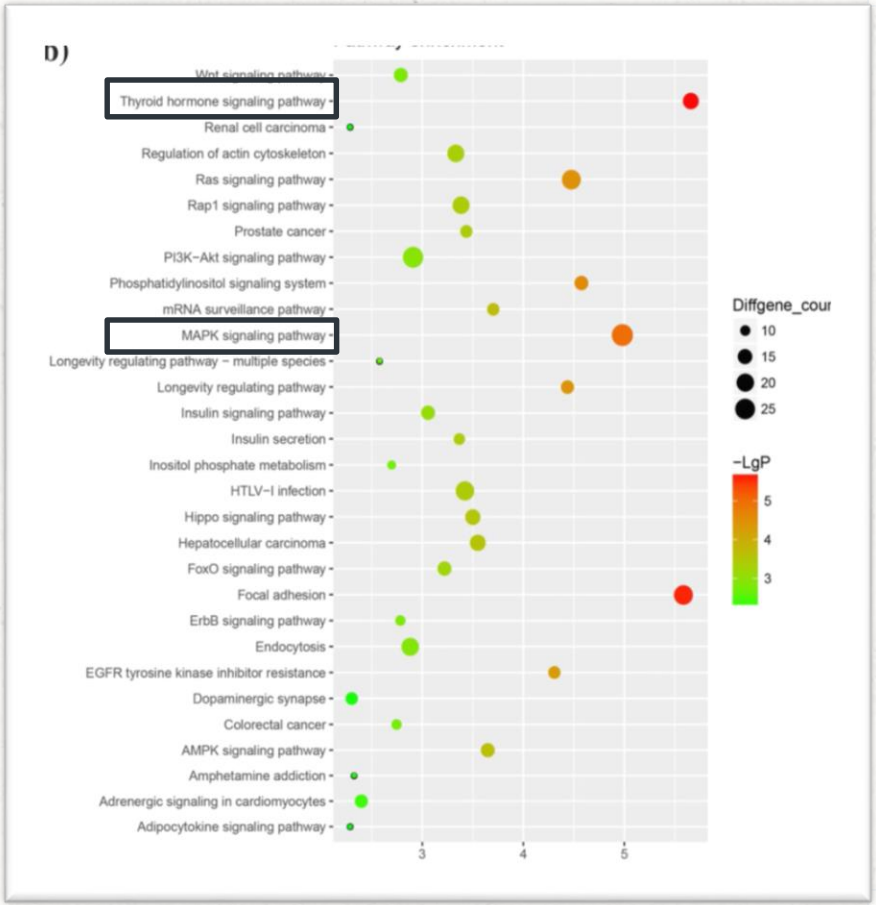

(a)
RNA polymerase II transcription cofactor activity
chromatin DNA binding
transcription factor activity, RNA polymerase II transcription factor ...
transcriptional activator activity, RNA polymerase II core promoter pr...
transcriptional activator activity, RNA polymerase II transcription re...
RNA polymerase II core promoter proximal region sequence−specific DNA ...
core promoter proximal region sequence−specific DNA binding
core promoter proximal region DNA binding
transcription coactivator activity
transcription factor activity, RNA polymerase II core promoter proxima...
presynaptic active zone
presynaptic membrane
phosphatase complex
protein serine/threonine phosphatase complex
histone deacetylase complex
occluding junction
nuclear envelope
nuclear membrane
presynapse
axon
negative regulation of neurogenesis
negative regulation of nervous system development
negative regulation of cell development
peptide hormone secretion
hormone secretion
forebrain development
hormone transport
signal release
axonogenesis
regulation of protein serine/threonine kinase activity
0
1
2
3
4
5
type
biological pathwa
cellular compone
molecular functio

5538



✓ Thyroid Hormone





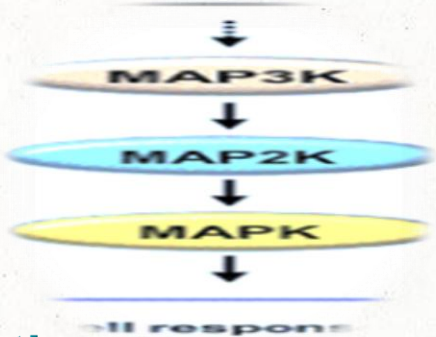
✓ **MAPK Signaling Pathway**

Innate Immunity

Inflammation

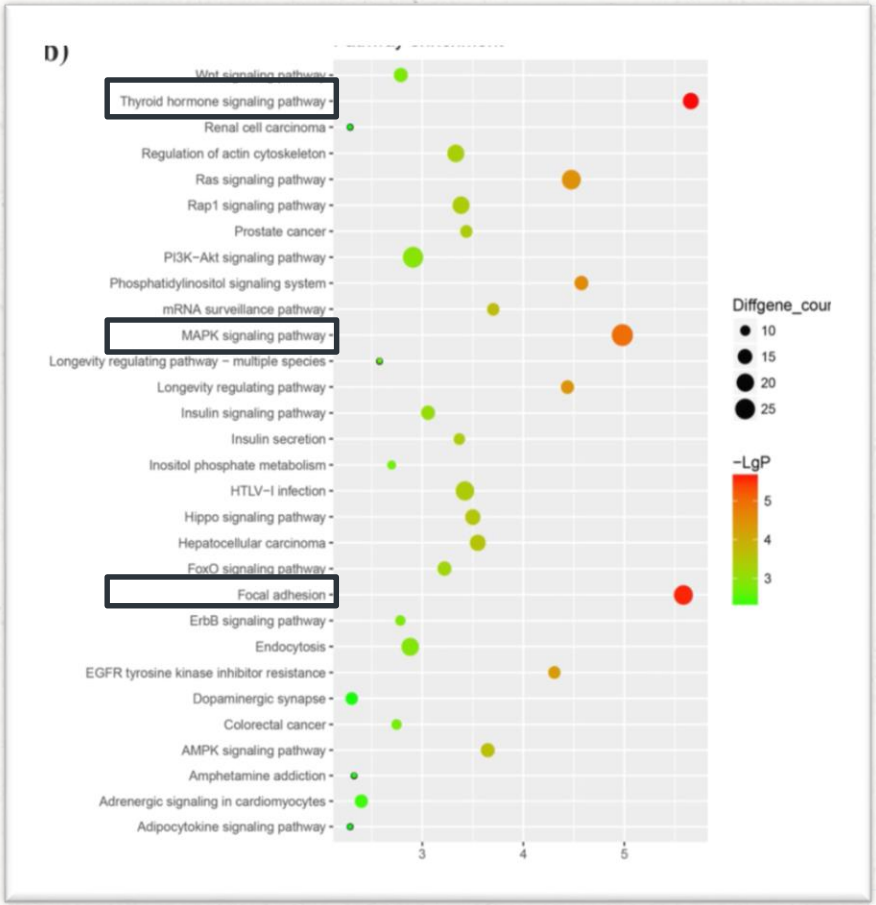
Apoptosis

Cell Growth



Cell Differentiation

Tumor Invasion and Metastasis

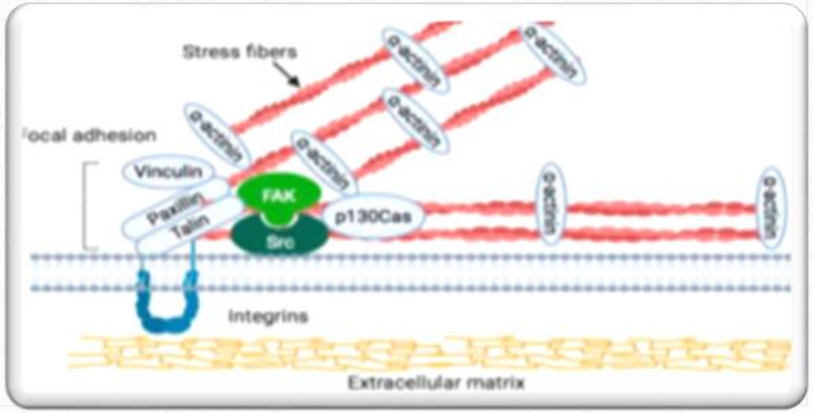


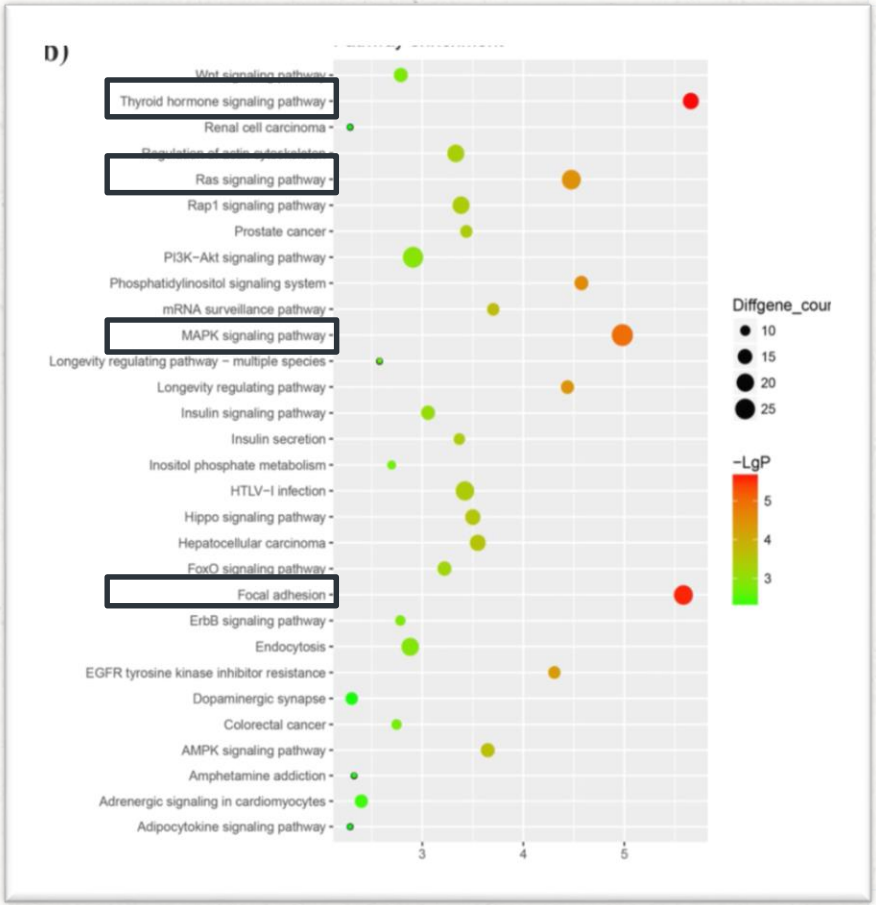
✓ **Focal Adhesion**

Cell Proliferation

Regulation of Gene Expression

Signaling Pathways





✓ **Ras Protein**

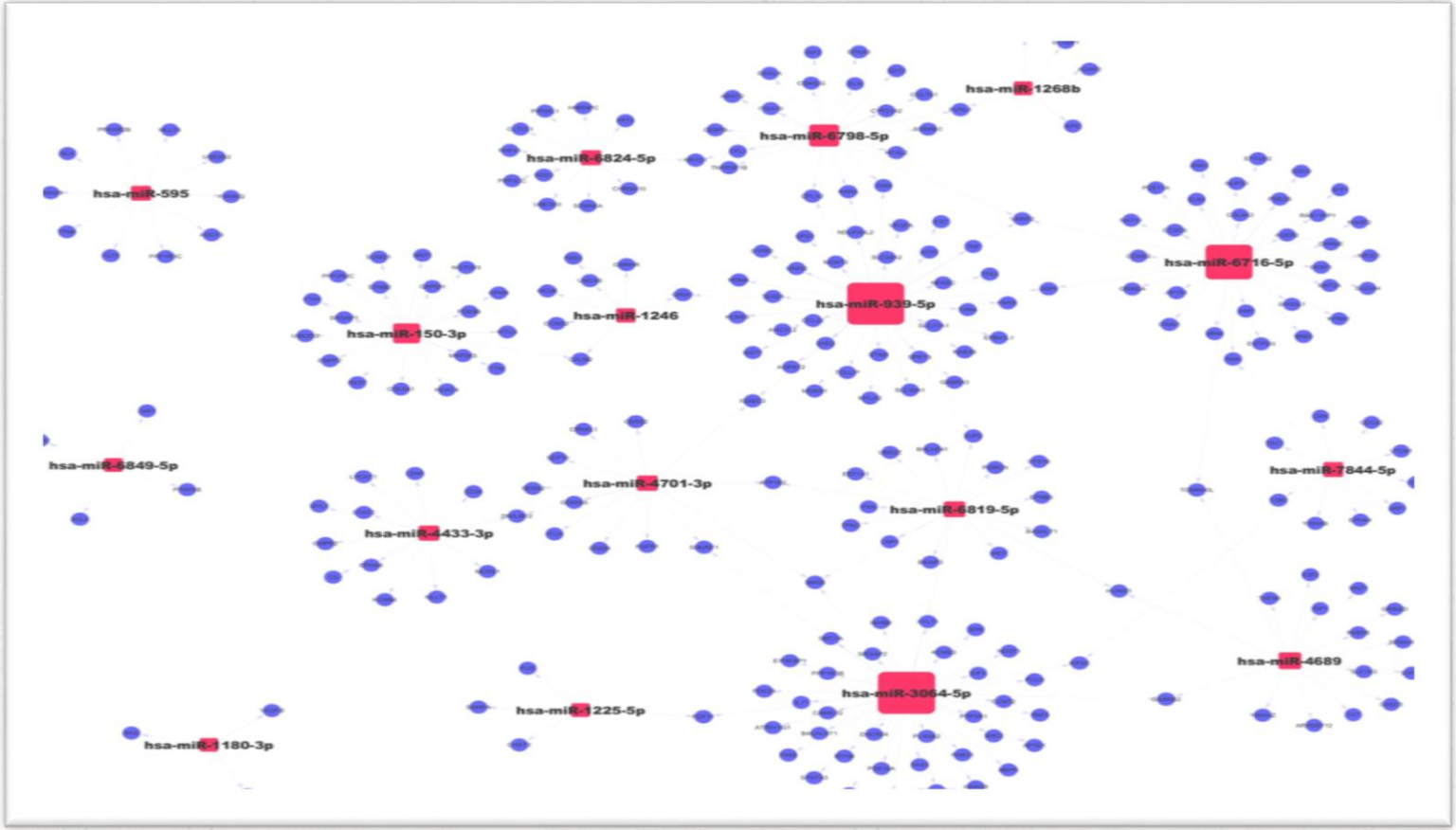
Cell Signaling Pathway

Regulating Cell Differentiation

Proliferation

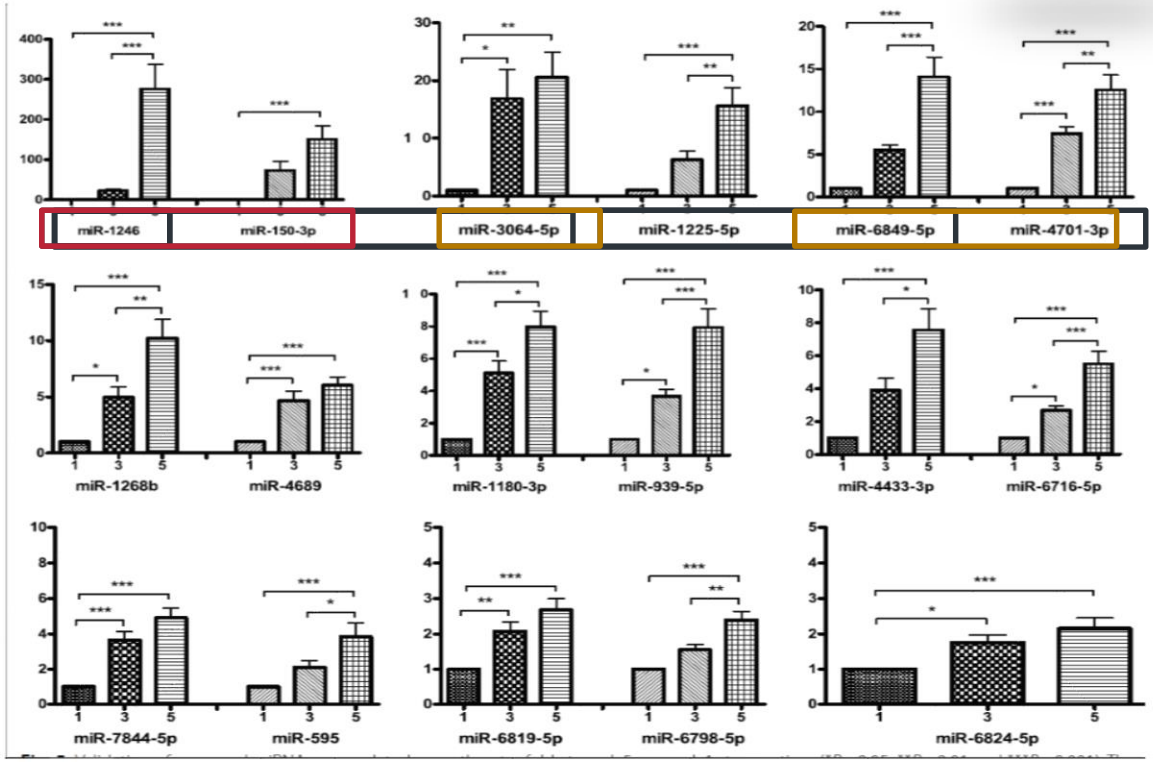
Apoptosis and Survival

Formation and Secretion of Exosomes


hsa-miR-1268b
hsa-miR-6798-5p
hsa-miR-6824-5p
hsa-miR-595
hsa-miR-6716-5p
hsa-miR-939-5p
hsa-miR-1246
hsa-miR-150-3p
hsa-miR-5849-5p
hsa-miR-7844-5p
hsa-miR-4701-3p
hsa-miR-6819-5p
hsa-miR-6433-3p
hsa-miR-4689
hsa-miR-3064-5p
hsa-miR-1225-5p
hsa-miR-1180-3p

6

CD4 + T cells

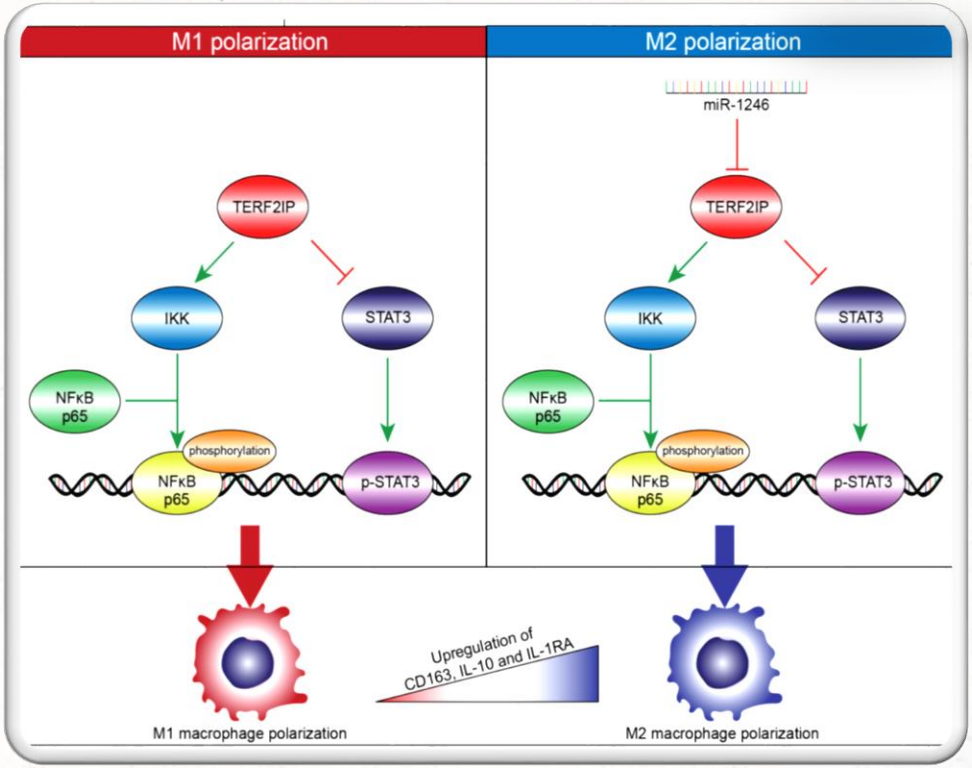

miR-1246
miR-150-3p
miR-3064-5p
miR-1225-5p
miR-6849-5p
miR-4701-3p
miR-1268b
miR-4689
miR-1180-3p
miR-939-5p
miR-4433-3p
miR-6716-5p
miR-7844-5p
miR-595
miR-6819-5p
miR-6798-5p
miR-6824-5p

MIRNA-1246

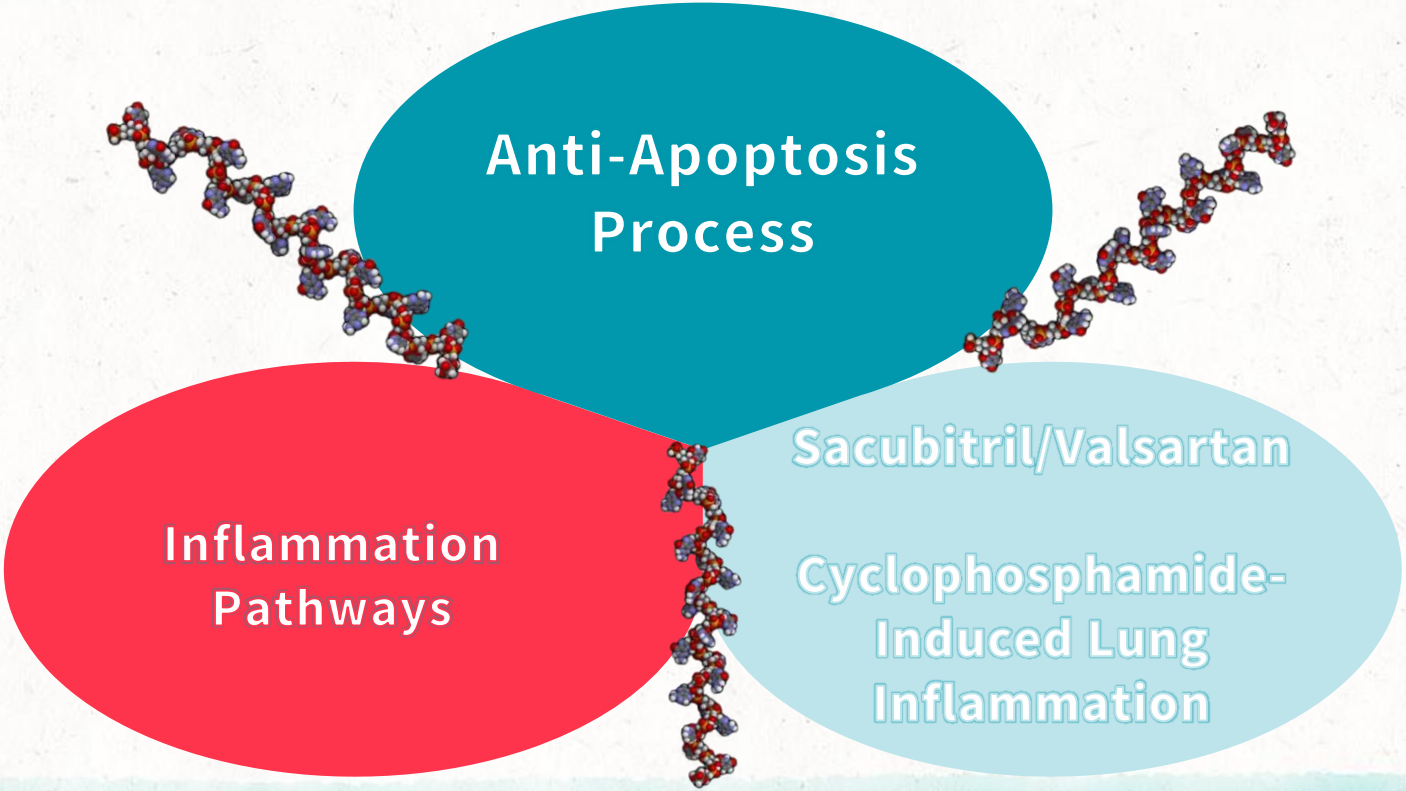
(LPS)-Induced Lung Injury

Neutrophil Activation

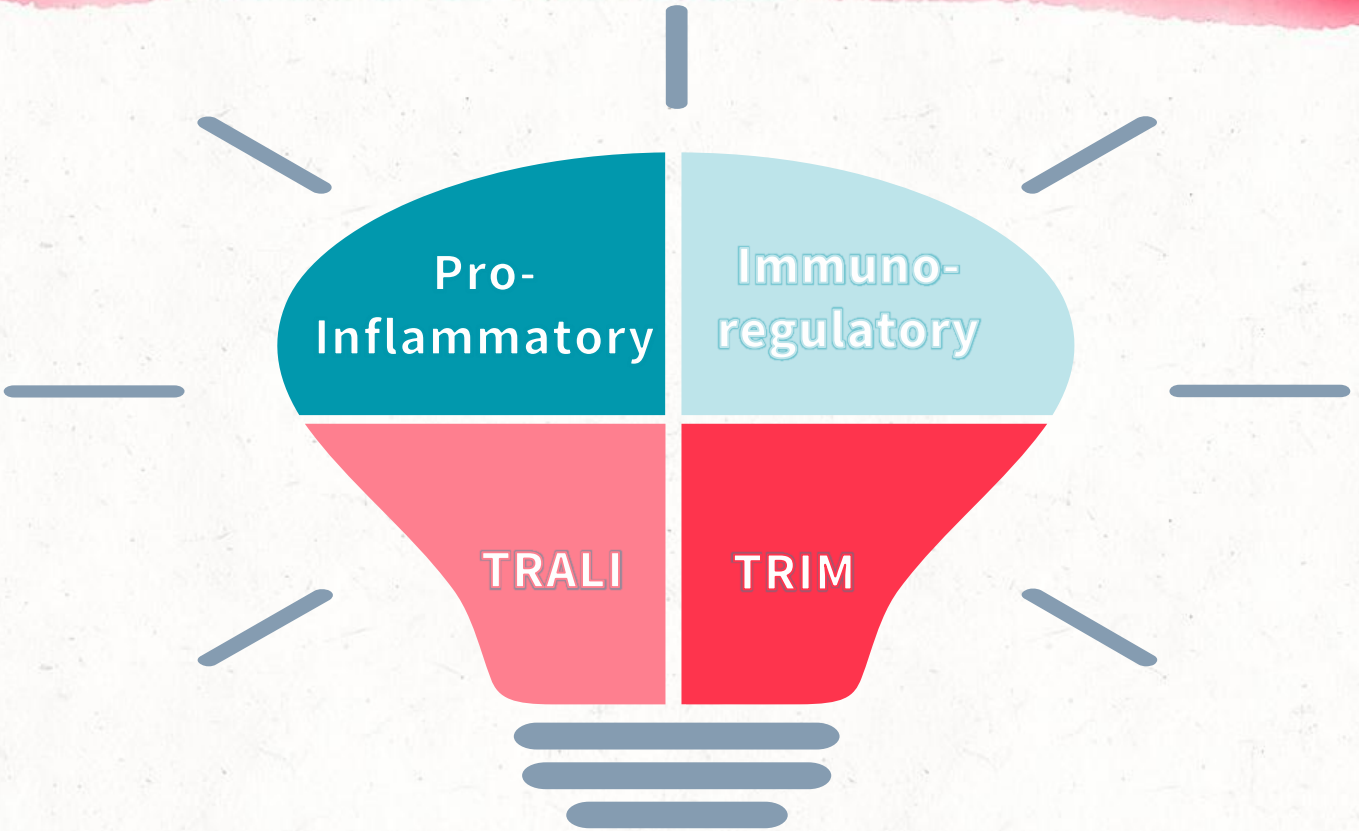
ACE2



MiRNA-150-3p


Anti-Apoptosis Process
Inflammation Pathways
Sacubitril/Valsartan
Cyclophosphamide-Induced Lung Inflammation

3.
CONCLUSION


Pro-
Inflammatory
Immuno-
regulatory
TRALI
TRIM


Thank
you