

Exosomes in NSCLC: differential cargo between 2D and 3D cultures.

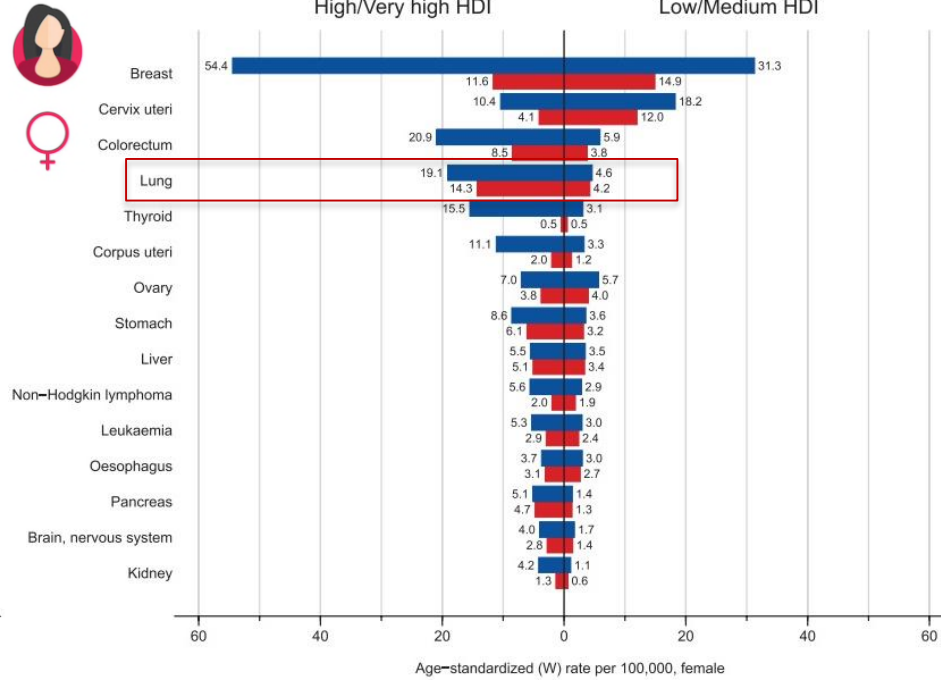
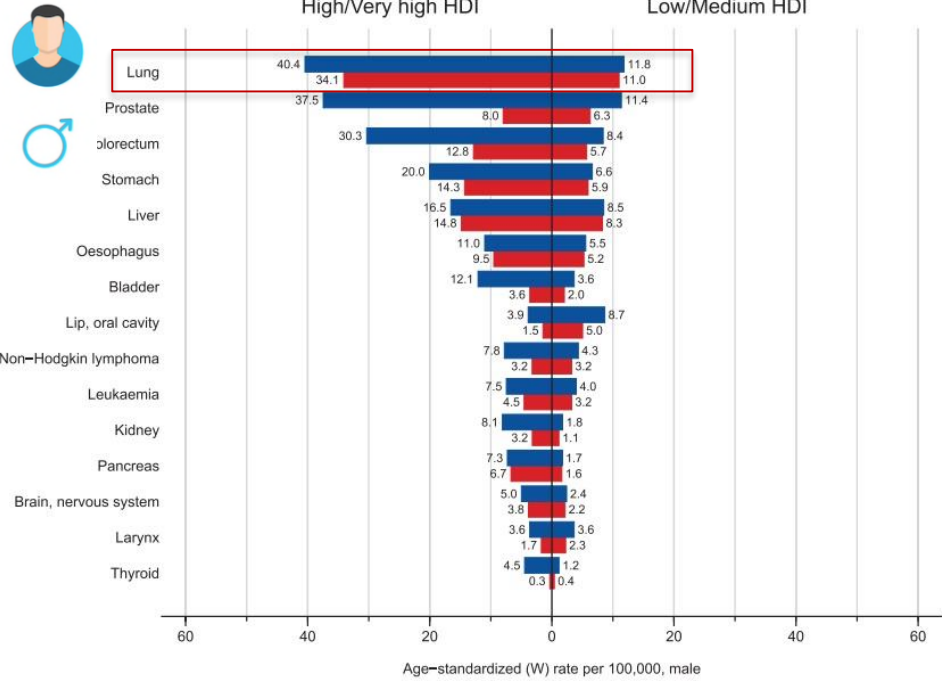
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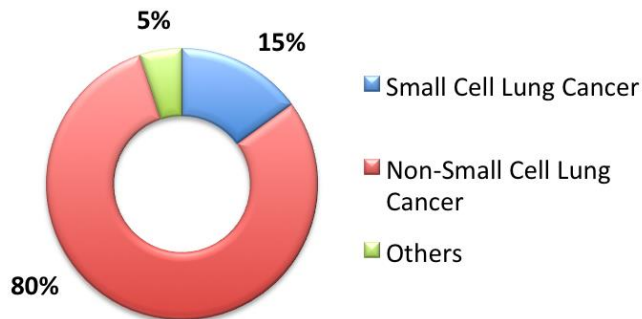


LUNG CANCER

INTRODUCTION



Lung Cancer Classification

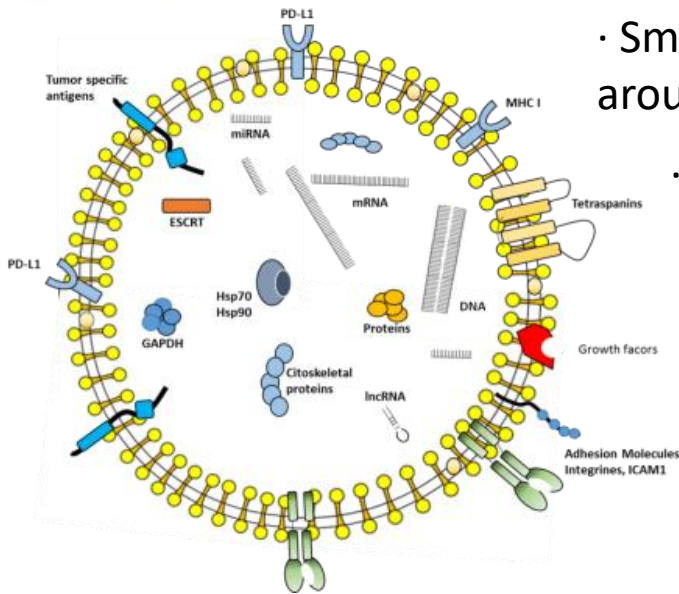


Incidence Mortality

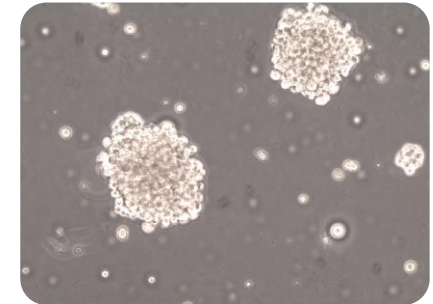
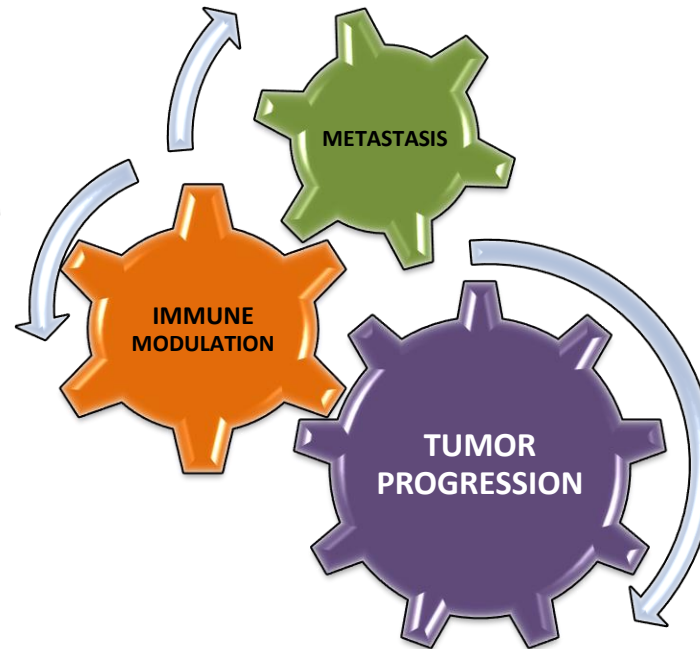
High/Very-High Human Development Index (HDI) Regions
vs.
Low/Medium HDI Regions among men and women worldwide
(2018)

INTRODUCTION + OBJECTIVES

- Small membranous vesicles around 40-130 nm
- May play a key role in NSCLC



CSCs-derived EXOSOMES

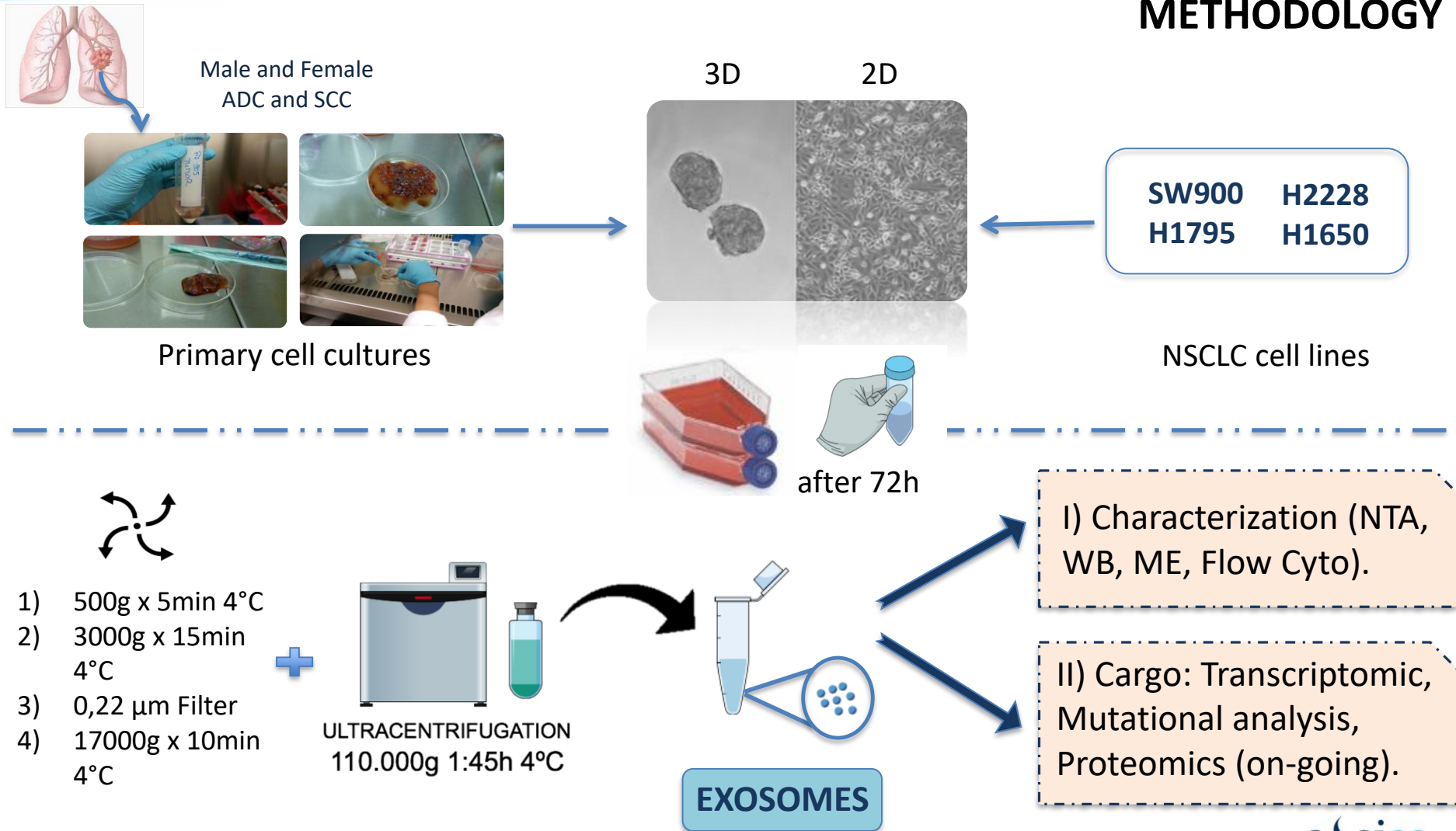


LUNG TUMORSPHERES
(3D CELL CULTURE) WITH
CANCER STEM CELLS (CSCs)
PROPERTIES



Objectives: Analyze the differences in exosomal cargo between lung tumorspheres (3D cell culture) and more differentiated tumor cells (2D).

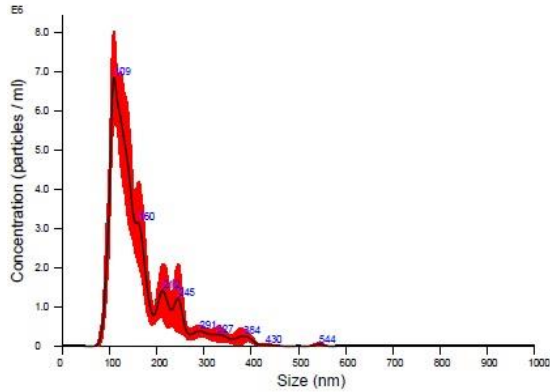
METHODOLOGY



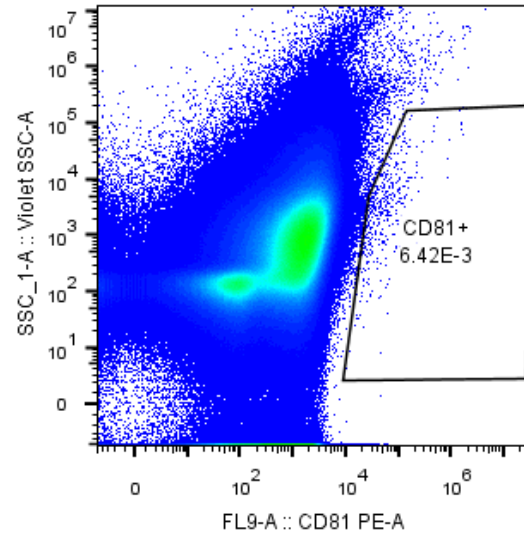
RESULTS (I): EXOSOMES CHARACTERIZATION

NTA

(Nanovesicle Tracking Analysis)



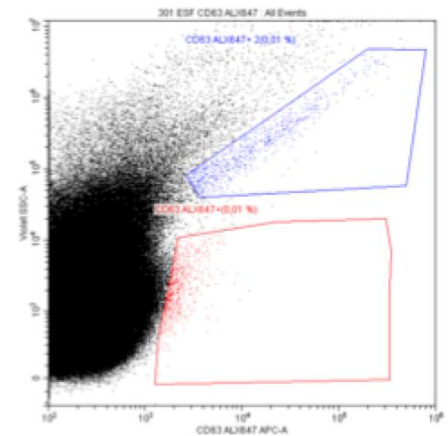
Range of exosomes size: 95 – 129 nm



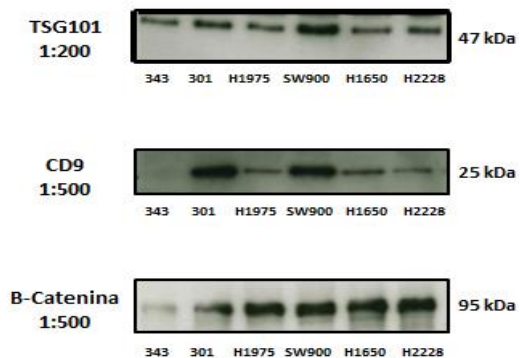
CD81+

FLOW CYTOMETRY

CD63+

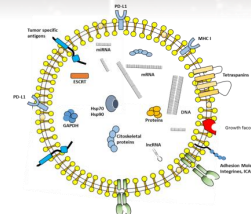
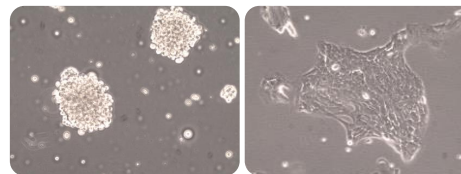


WESTERN BLOT

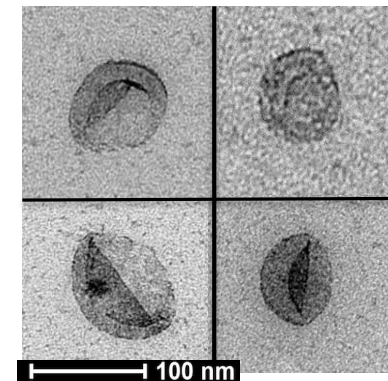


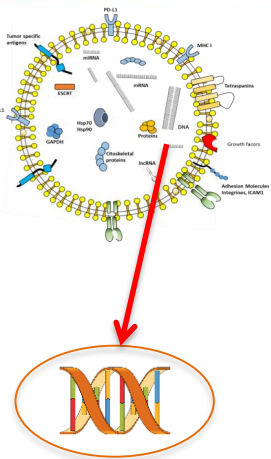
3D

2D



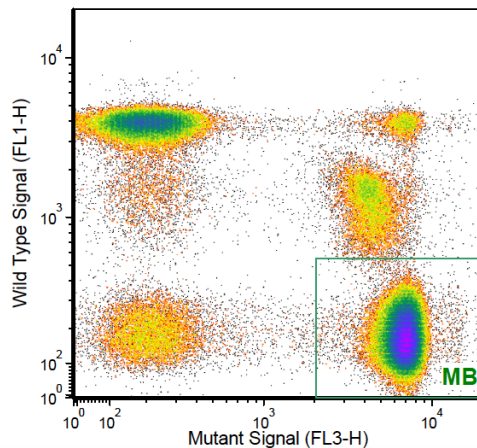
ELECTRON MICROSCOPY





	SW900	H1975	H1650	H2228	PAT. 343	PAT. 301
GENDER	MALE	FEMALE	MALE	FEMALE	FEMALE	MALE
HISTOLOGY	Squamous cell carcinoma (SCC)	Adenocarcinoma (ADC)	Adenocarcinoma (ADC)	Adenocarcinoma (ADC)	Adenocarcinoma (ADC)	Squamous cell carcinoma (SCC)
MUTATIONS	KRAS CODON 12 (G12V)	EGFR (L858R, T790M)	EGFR (EXON 19 DELETION)	ALK+ RB1 p.E204fs*10	NO	NO

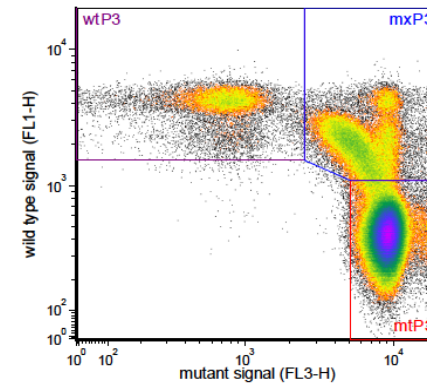
Wild type vs. mutant beads



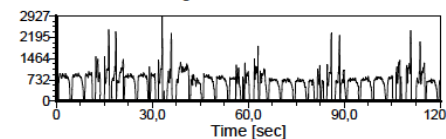
Parameter	Value	Pass/ FAIL
SB# (AP1)	714115	Pass
SBpos (AP2)	642 / 677	Pass
IP1% (AP3)	0,37%	Pass
Extended Beads (EB#)	135884	Pass
Extension Rate (ER)	19,03%	Pass
Mutant Beads (MB#)	75186	
Mutant Fraction (MF)	55,3310%	

EGFR MUTATIONS

Wild type vs. mutant beads



Single beads over time



Parameter	Pass / FAIL
AP1 (SB#)	Pass
AP2 (SBpos)	Pass
AP3 (IP1%)	Pass
AP4 (EB#)	Pass
AP5 (EB%)	Pass
AP6 (IP2%)	n.a.
AP7 (IIP2%)	n.a.
AP8 (IIIP2%)	n.a.
AP9 (EB%IIP2%)	Pass
AP10 (wtP3pos)	Pass
AP11 (wtP3CV)	n.a.
AP12 (mtP3pos)	Pass
AP13 (mtP3CV)	n.a.
AP14 (mutant beads)	259434
AP15 (mutant fraction)	79,127%
MVC Valid?	PASS
Test Status	mutation detected

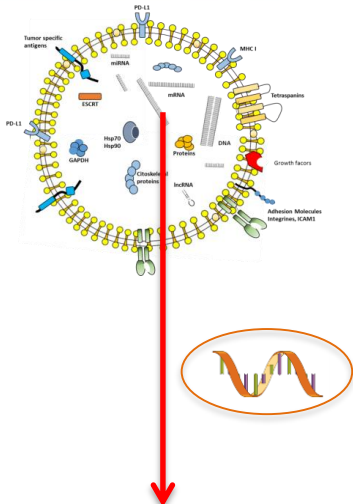
Please see Retest section of OncoBEAM® RAS CRC Kit Instructions for Use for retesting guidance for failed parameters

RAS MUTATIONS

EXOSOMAL DNA
(Most common mutations detection in NSCLC)



BEAMing Digital PCR technology



EXOSOMAL RNA



Collaboration:
J.Paramio
(CIEMAT)

Clariom D Human Assay
>540.000 transcripts:
Genes, exons, alternative
splicing, coding and non-coding
RNA isoforms

5.998 differentially expressed
transcripts

Other RNA isoforms, Non-
codings, splice variants, **miRNAs**
and precursors

1.193 differentially expressed
genes (mRNA)

25 genes in common with other
subcategories (histology, etc.)

**1.168 differentially
expressed genes (mRNA)
only in 3D vs 2D group**

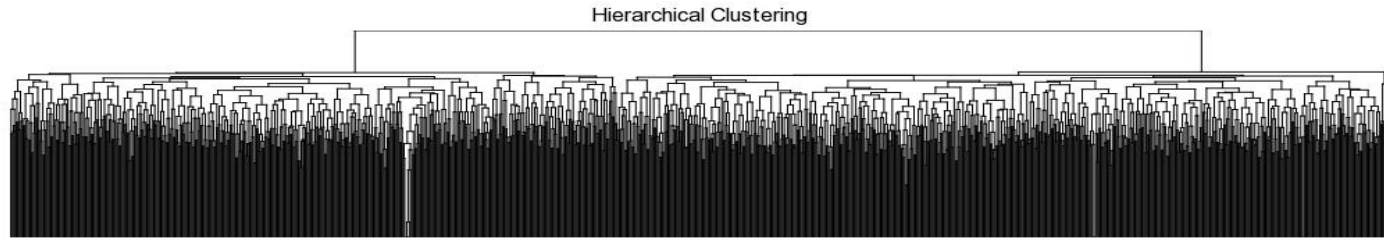
Pathway enrichment
analysis

p-value: < 0.05

**28 differentially expressed
miRNAs and precursors in
3D vs 2D group**

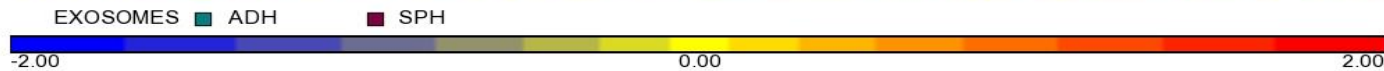
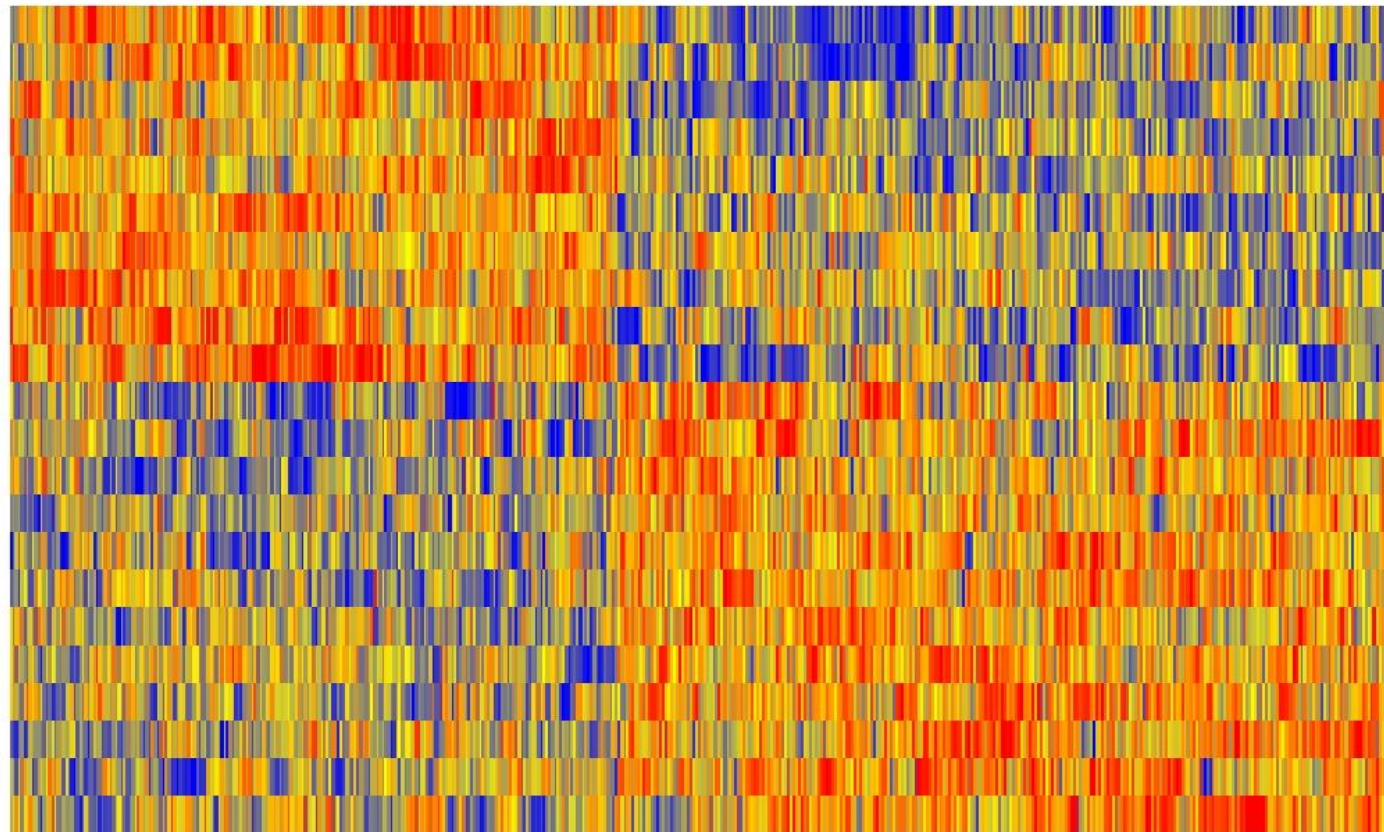
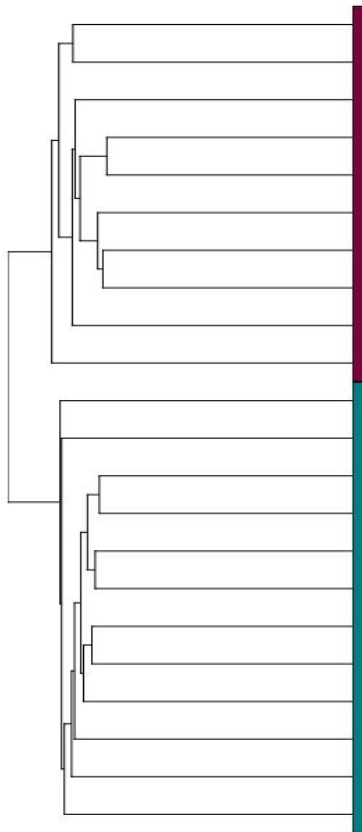
Pathway enrichment
analysis

DIFFERENTIALLY
EXPRESSED GENES
(mRNA)



3D-DERIVED EXOSOMES

2D-DERIVED EXOSOMES



Pathways overexpressed in 3D-derived exosomes

METHOD	GO ID	DESCRIPTION	p-value
Enrichr	GO: 1905475	Regulation of protein localization to membrane	0.010
Enrichr	GO: 0014808	Release of sequestered calcium ion into cytosol by sarcoplasmic reticulum	0.012
Enrichr	GO: 0015879	Carnitine transport	0.016
Enrichr	GO: 0071415	Cellular response to purine-containing compound	0.020

Pathways overexpressed in 2D-derived exosomes

METHOD	GO ID	DESCRIPTION	p-value
Enrichr	GO: 0007031	Peroxisome organization	0.006
Enrichr	GO:0033262	Regulation of nuclear cell cycle DNA replication	0.019
Enrichr	GO:0010310	Regulation of hydrogen peroxide metabolic process	0.019
Enrichr	GO:0034446	Substrate adhesion-dependent cell spreading	0.021

✧ Summary of the results of the pathway enrichment analysis in the differentially expressed 3D vs 2D mRNA group.

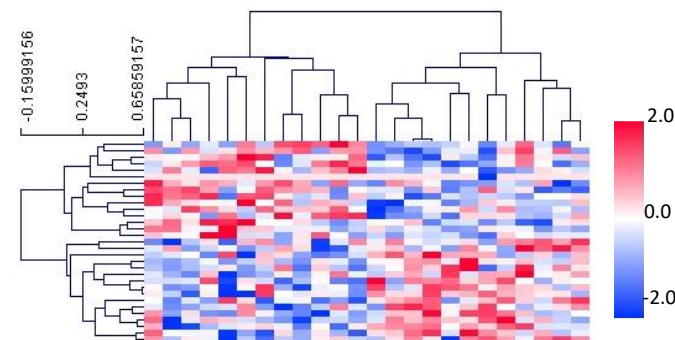
28 miRNAs and precursors
expressed in
3D vs 2D-derived exosomes



TARGETS
IDENTIFICATION

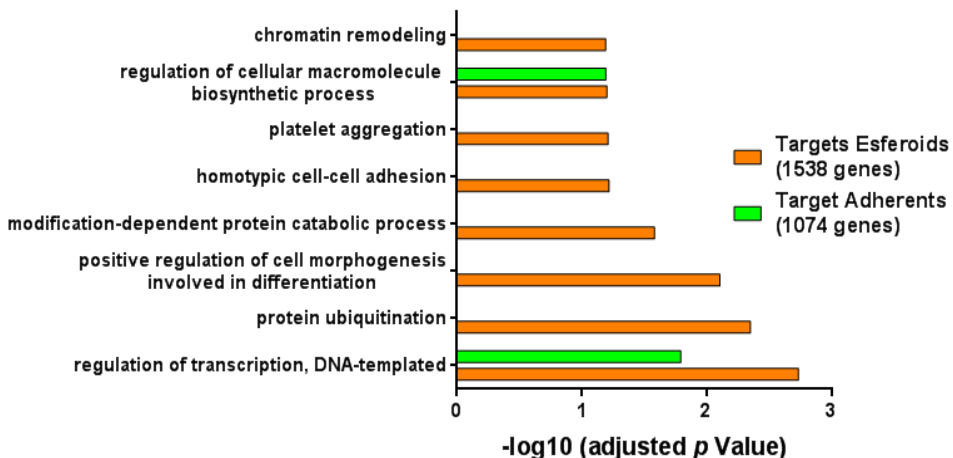


Spheroids (3D) Adherents (2D)

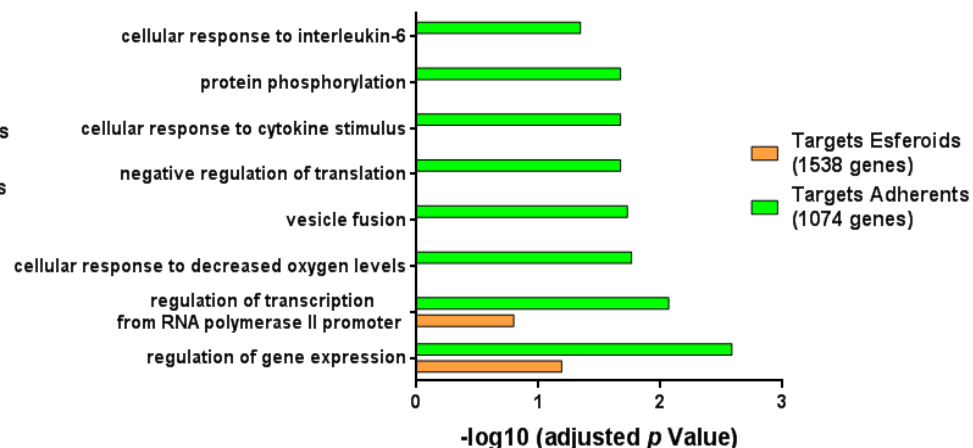


GOBP category

Targets unique Esferoids



Adherents



✧ Summary of the results of the pathway enrichment analysis in the differentially expressed 3D vs 2D miRNA and precursors group.

CONCLUSIONS

Differences in the cargo of exosomes have been observed between lung-tumourspheres (3D) and more differentiated tumor cells (2D).

- ✧ Exosomal cargo may reflect the molecular signatures of the cells from which they were secreted → Good source for biomarkers analysis.

ONGOING...

3D vs 2D Cargo : Proteomics.

Analysis and characterization of plasma exosomes from NSCLC patients.

16th ASEICA INTERNATIONAL CONGRESS

Valencia, 6th - 7th - 8th November 2018



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