UNIVERSITÀ DEGLI STUDI DI TRIESTE

Dipartimento di Scienze della Vita

Exosomal circLPAR1 functions in colorectal cancer diagnosis and tumorigenesis through suppressing BRD4 via METTL3-eIFh3 interaction

Rui Zheng, Ke Zhang, Shanyue Tan, Fang Gao, Yajie Zhang, Wenxia Xu, Huabin Wang, Dongying Gu, Lingjun Zhu, Shuwei Li, Haiyan Chu, Zhengdong Zhang, Lingxiang Liu Mulong Du and Meilin Wang

Presented by Petra Ravalico and Alice Zat

Outline

Introduction: circRNAs

CircLPAR1: experimental results

CircLPAR1: discussion

Conclusions

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Introduction: circRNAs

CircLPAR1: experimental results

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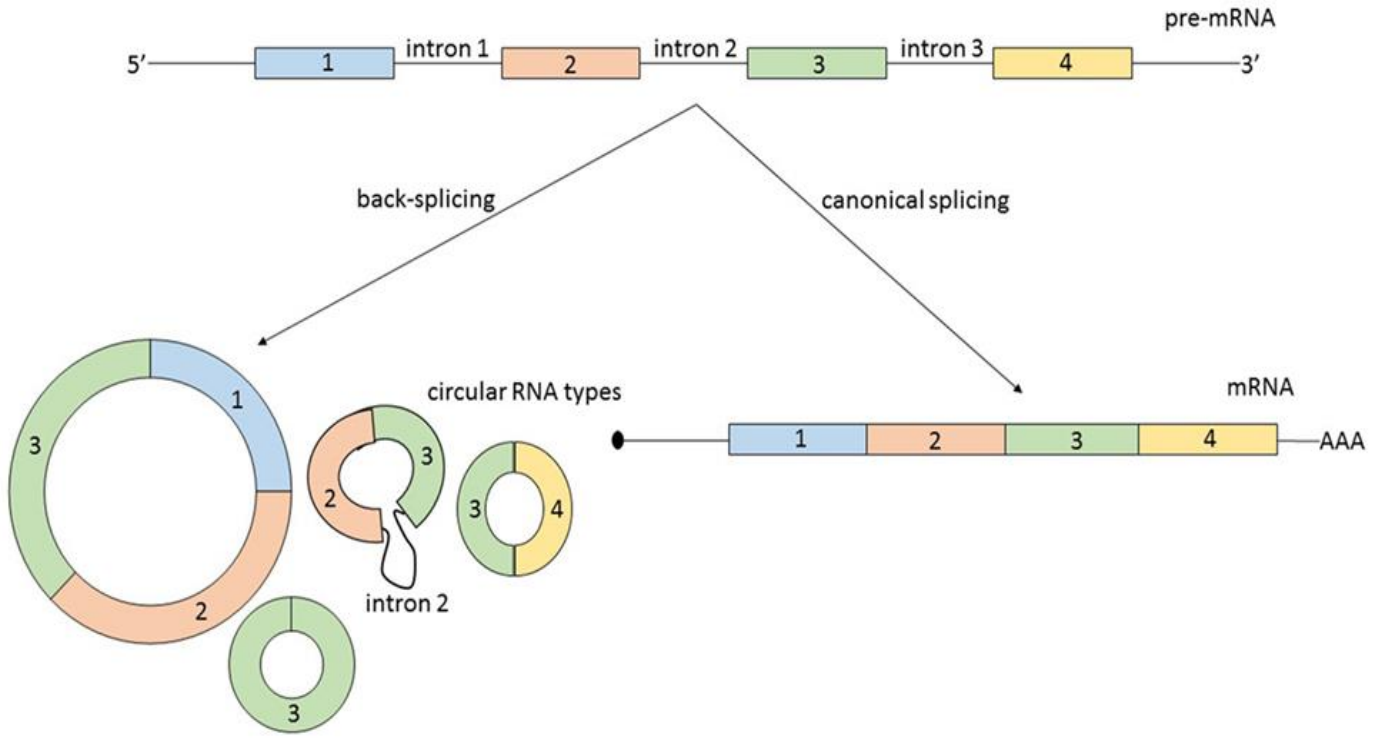
Conclusions

Circular RNAs – characteristics

→ RNA **single-stranded** molecule, covalently closed, forming a **loop structure**

CircRNAs are synthesized by **back-splicing** → covalent interaction between 3’ and 5’ splice sites

Highly stable structure due to the lack of 3’ and 5’ free ends → no ribonuclease degradation

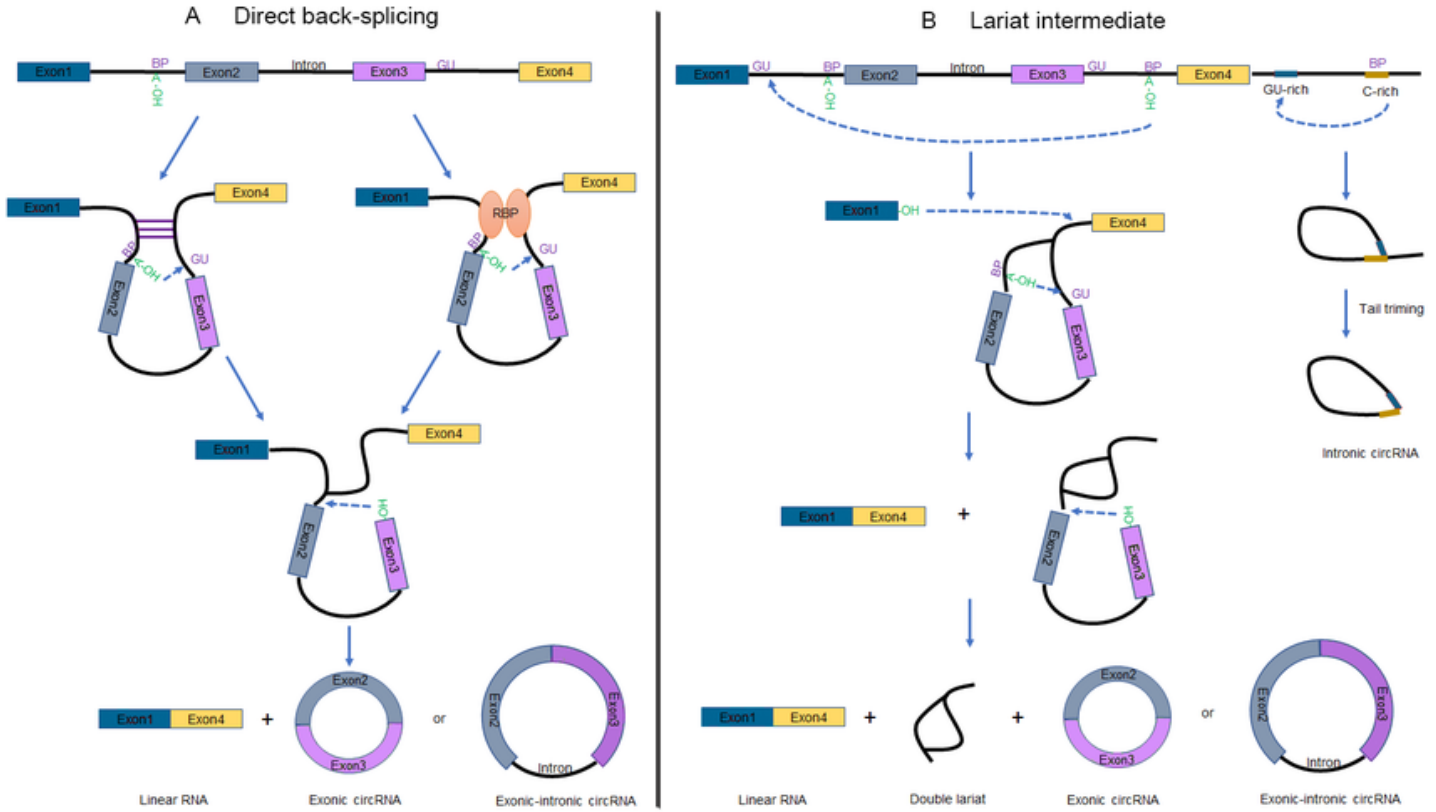


[Dragomir et al., 2018]

Circular RNAs – back splicing

Back-splicing – alternative splicing process in which the donor splice site at the 5' end **covalently binds** to the acceptor splice site at the 3' end

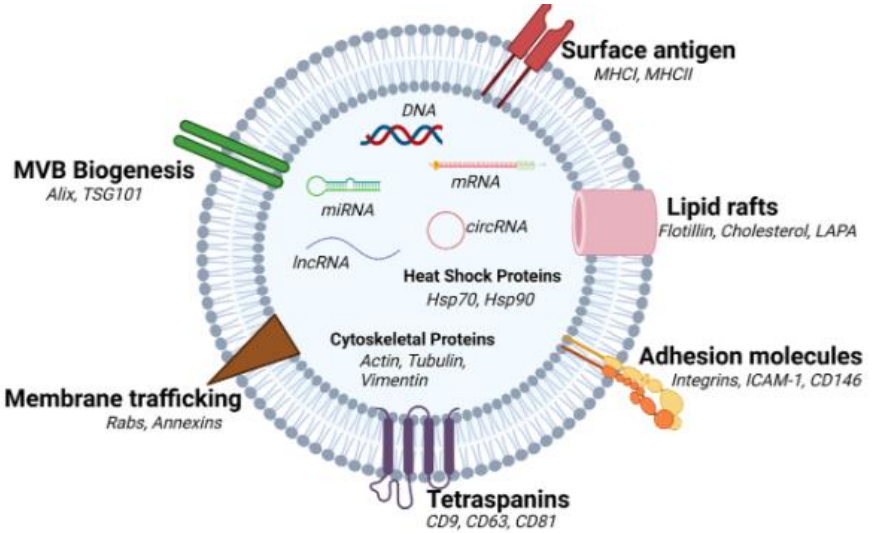
Two models are proposed:



[Li et al., 2020]

Circular RNAs – localization

- They are located both in the **nucleus** and **cytoplasm**
- **Tissue-specific** expression pattern
- They can be secreted by cells in **exosomes**



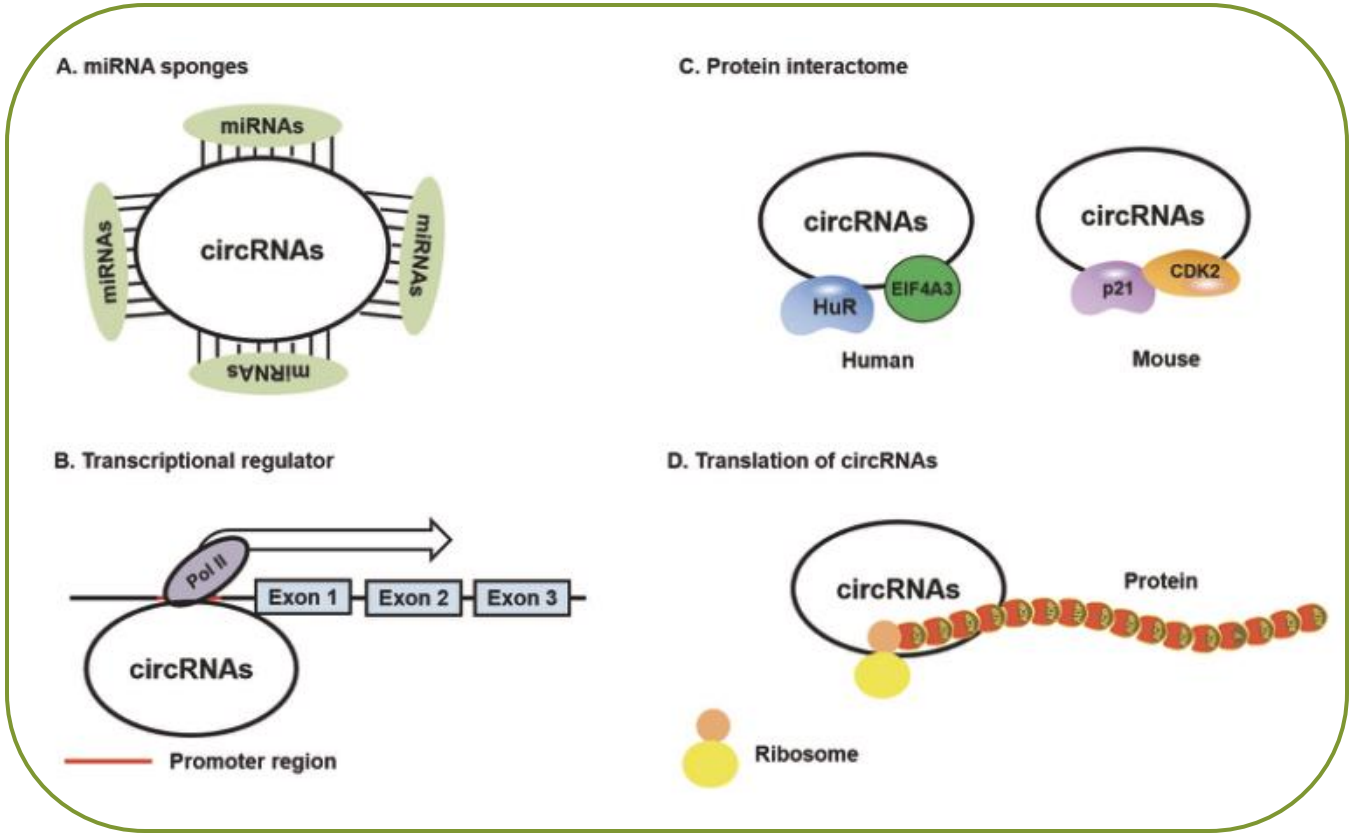
[Boussios et al., 2023]

Cell-to-cell communication, **all types** of cells can **generate** exosomes and **all types** of cells can **receive** them

Vesicles with phospholipid bilayer
Dimension: 30-150 nm
Localization: plasma and also in any **body fluids**
Content: proteins, growth factors, lipids, nucleic acids and **ncRNAs**

CircRNAs regulate gene expression

A. miRNA sponges
B. Regulation of transcription and translation
C. Protein sponges
D. ORF-containing circRNAs



[Zhang et al., 2023]

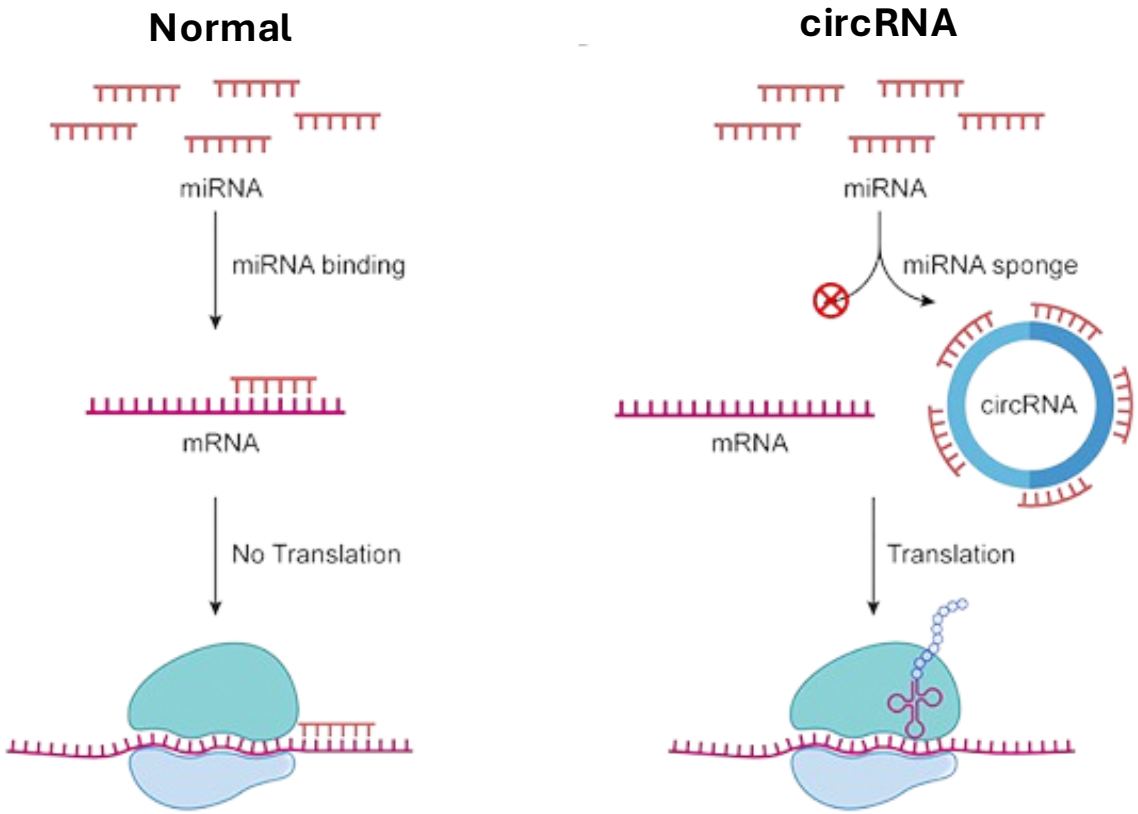
A. miRNA sponges

CircRNAs can behave as miRNAs sponges, limiting their action in **transcriptional** and **post-transcriptional** gene expression

Mechanism: circRNA → binds to miRNA → miRNA cannot bind to target mRNA → **increase of protein production**

Examples:

- circSHKBP1 in CRC – binds to miR-328-5p → increase of E2F1 (de-repression)
- circPACRGL in CRC – promotes cell proliferation, migration and invasion



[Min et al., 2021]

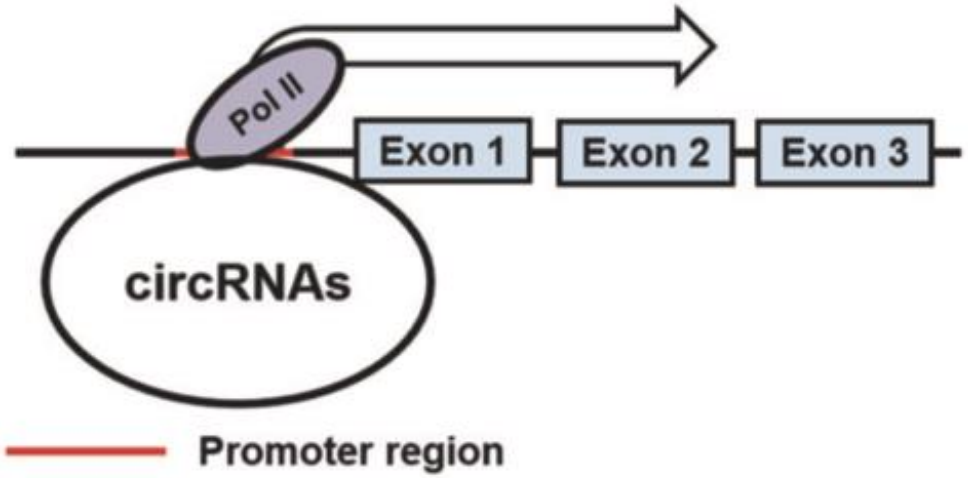
B. Regulation of transcription and translation

Regulation of gene transcription by **interacting with RNA polymerase II**:

- circEIFJ3 and circPAIP2 promotes the transcription of their own gene by interacting with U1 and RNA pol II

Others example:

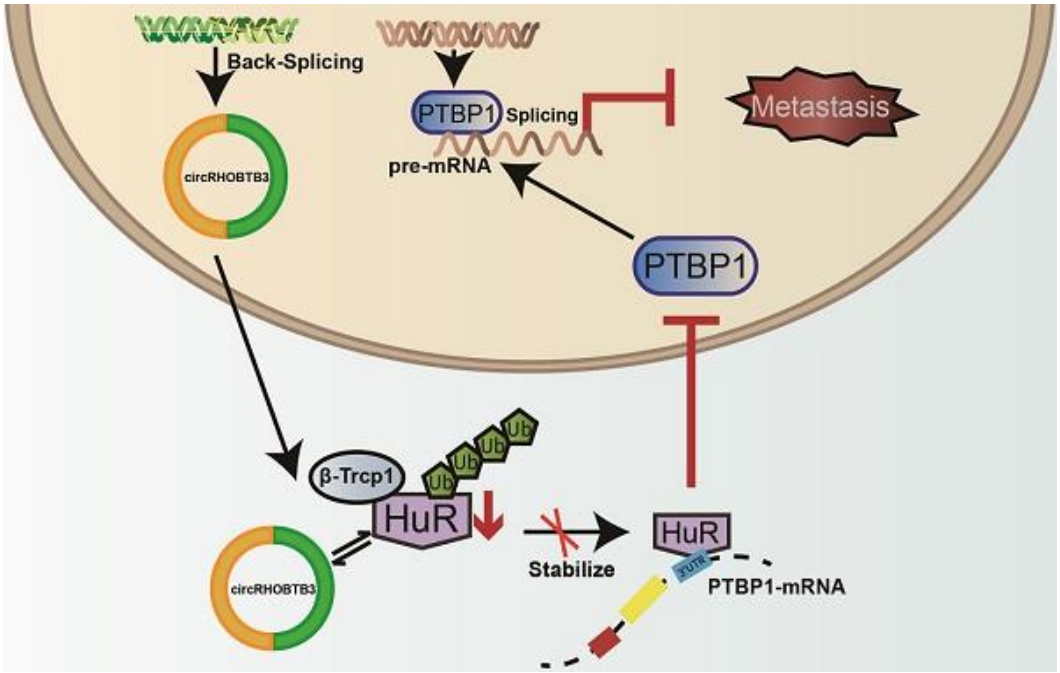
- circLPAR1 – **anti-tumorigenesis action** by interacting with eIF3h and inhibiting the interaction with METTL3
- circVAMP3 – **suppression of tumor growth** by reducing the protein levels of MYC



[Zhang et al., 2023]

C. Protein decoys

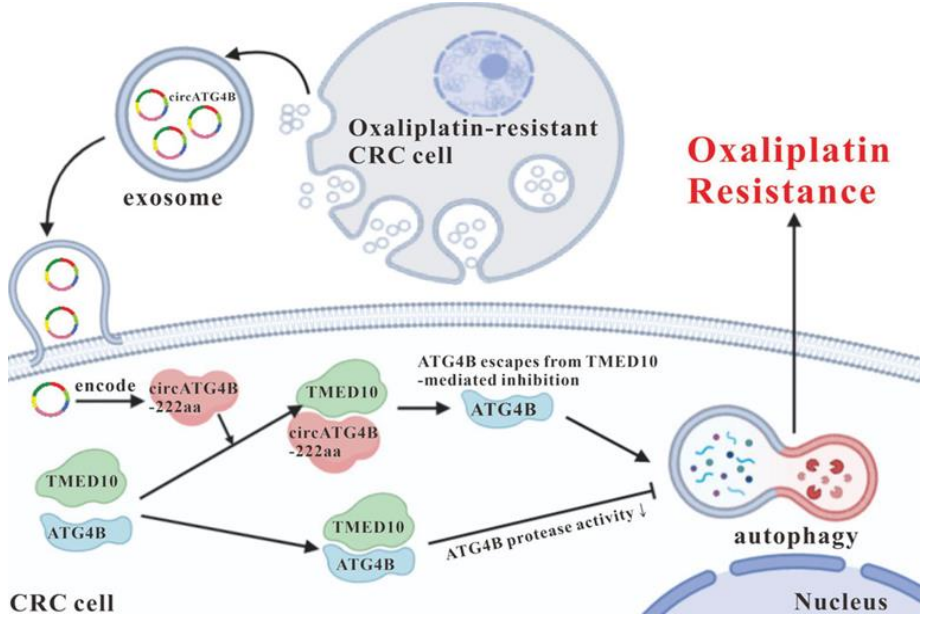
- RBP binding sites → **RNA:protein complexes**
- Diverse effects
- Ubiquitin-mediated **degradation**: circRHOBTB3 and HuR complex → metastasis suppression
- **Inhibition** of function: circPABPN1 and HuR inhibition → reduced autophagy (IBD)
- **Enhancing** interaction: ternary complex circFOXO3-CDK2-p21 → block of cell cycle



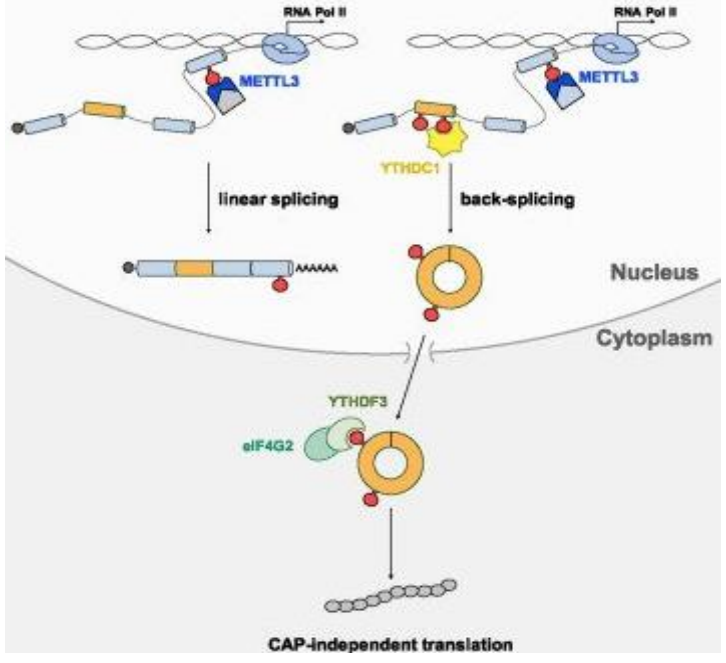
[Chen et al., 2021]

D. ORF-containing circRNAs

- Some circRNAs are **coding for proteins**
- **Tumour suppressing** property: *circPLCE-411* protein inhibits CRC proliferation and metastasis
- **Oncogenic** role: exosomal *CircATG4B* and oxaliplatin resistance



[Pan et al., 2022]



- Translation 1. IRES-*dependent* 2. IRES-*independent*
- **m^{6}A** epigenetic modification: YTHDF3 (reader) modulates *circZNF609* translation →myoblast proliferation

[Di Timoteo et al., 2020]

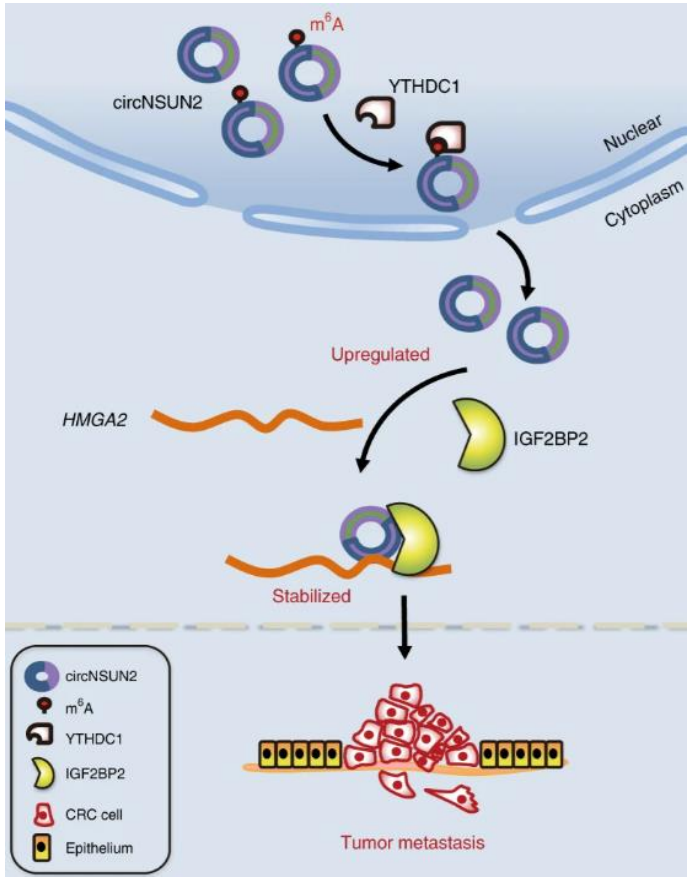
CircRNAs dysregulation

- Controlled levels → physiological roles
- Dysregulation → Cardiovascular and neurological disorders; cancer
- **Cancer type-specific** dysregulation

Hypothesis:

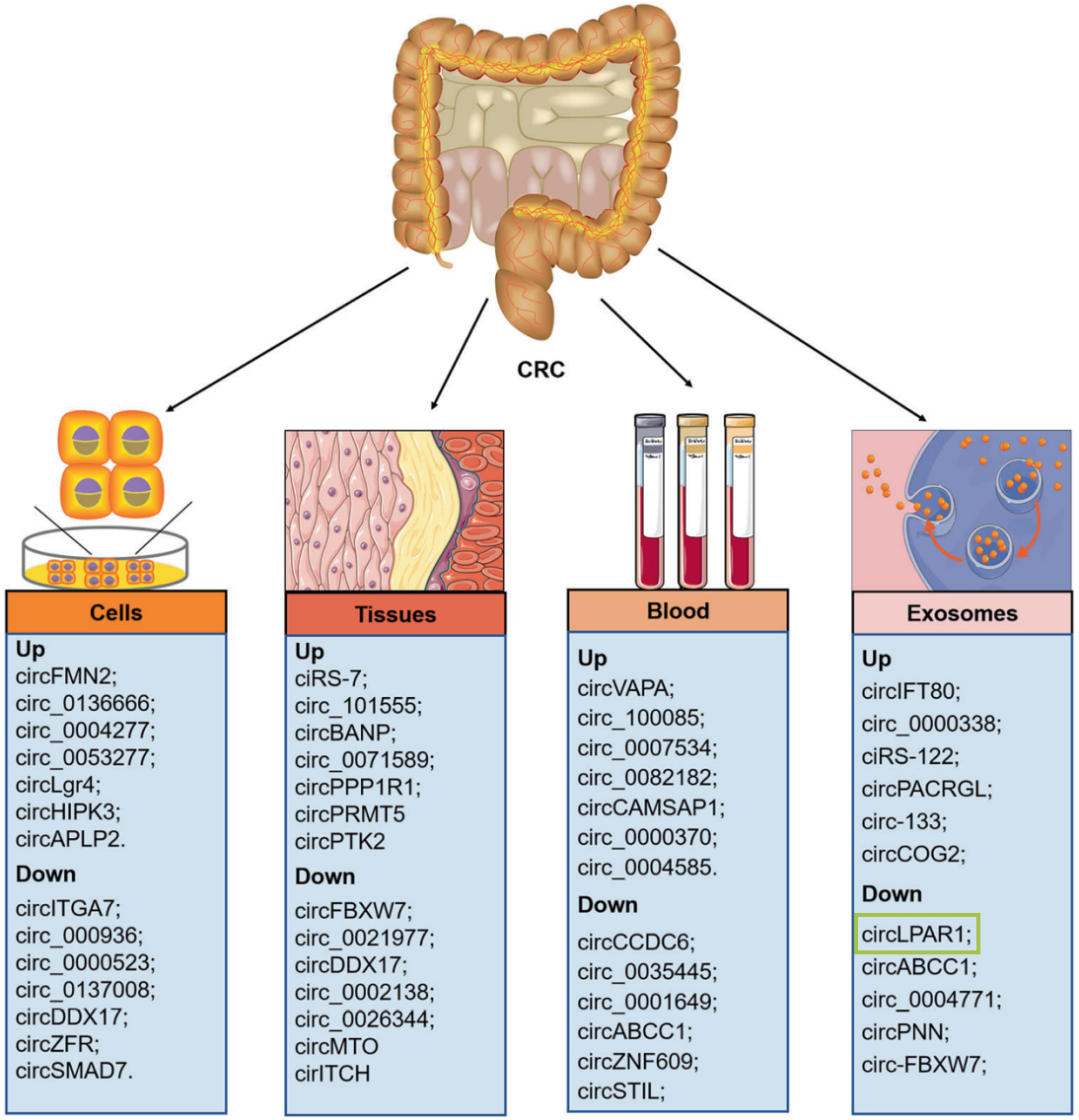
- Chromosomal aberrations: *fusion circRNAs* (ex: *FcircSR1-2* in NSCL carcinoma)
- Exocytosis: *exosomal circRNAs*
- **m^{6}A modification:** circRNAs degradation, expression, transport
- Super Enhancers control

ADAR1 as **negative regulator** of circRNA biogenesis



[Chen. et al, 2019]

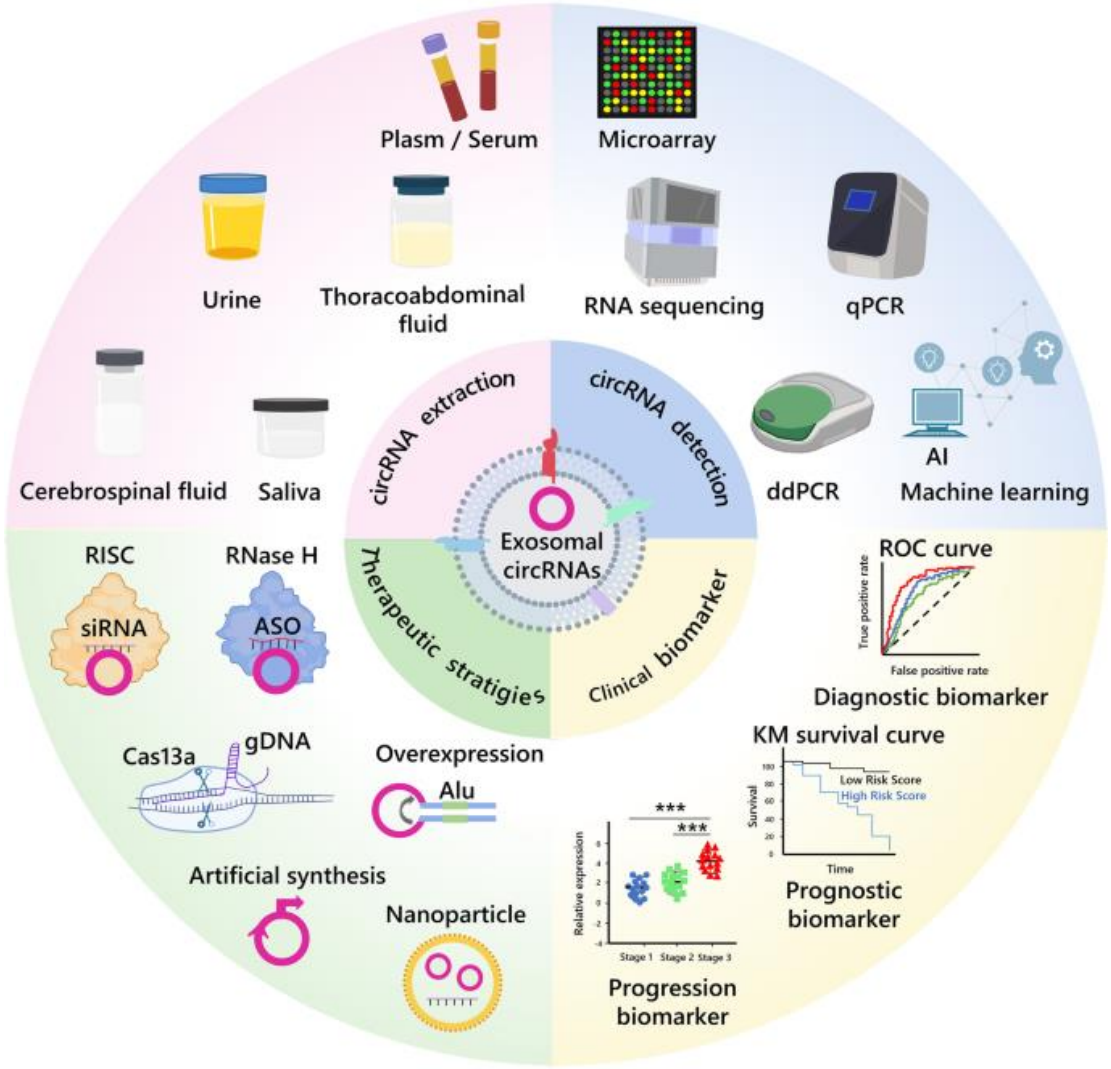
CircRNAs relevance in CRC



[Zhang et al., 2023]

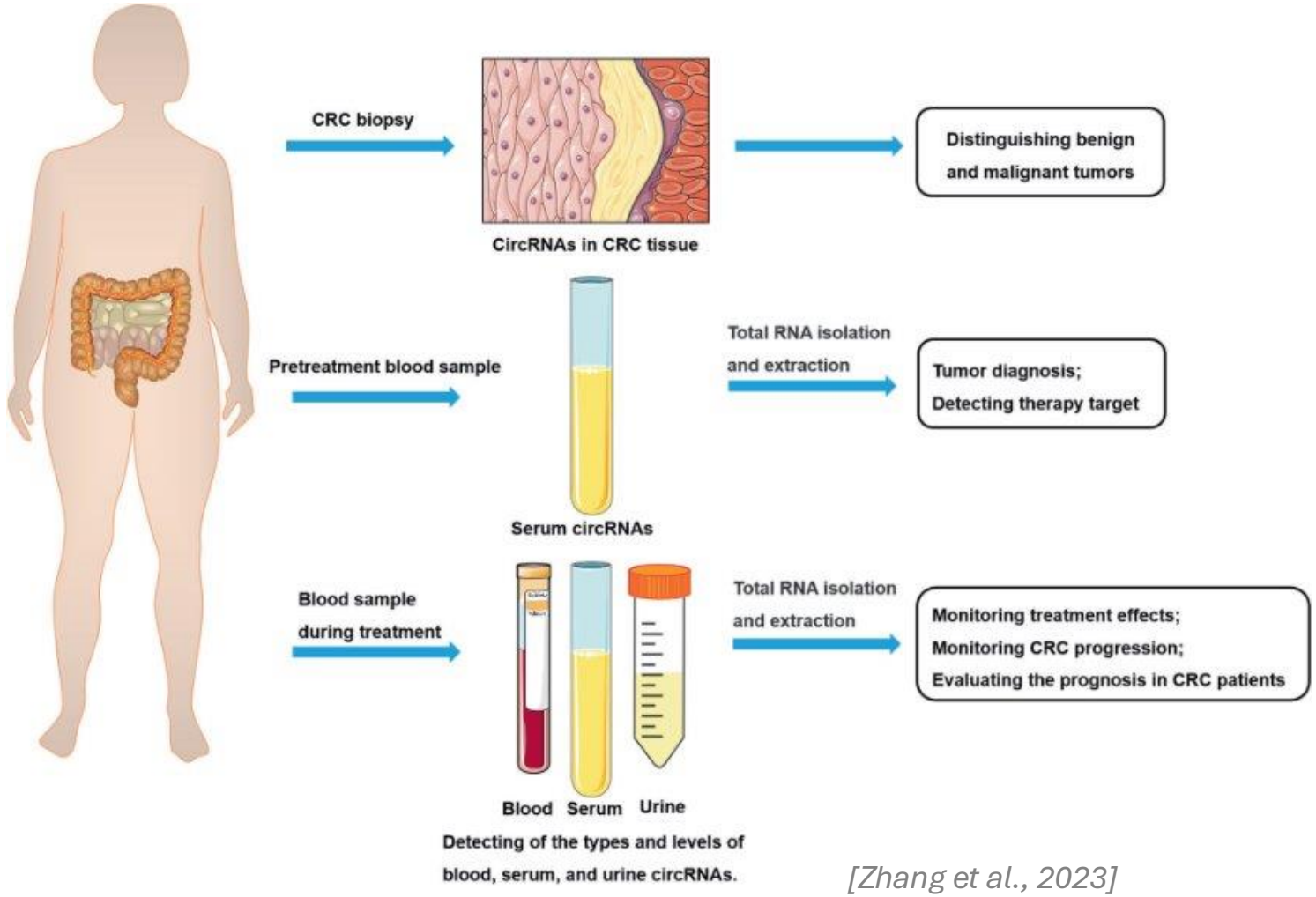
CircRNAs in the clinics

- ✓ **Stable**
- ✓ Highly **cancer type-specific**
- ✓ Present and **abundant** in body fluids and tissues
- ✓ **Inexpensive** and **non-invasive** analysis
- ✓ High specificity and sensitivity (some)
- ✓ Easily **detectable** (qRT-PCR or microarray)



[Wang et al,. 2024]

CircRNAs in the clinics



[Zhang et al., 2023]

Useful as:

- **Diagnostic** biomarker: aberrant expression → clinical implications
- Key players in many cancer signalling pathways → **targets** for RNA therapeutics
- **Prognostic** biomarker: chemoresistance and metastasis

Outline

Introduction: circRNAs

CircLPAR1: experimental results

CircLPAR1: discussion

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Zheng *et al. Molecular Cancer* (2022) 21:49
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Molecular Cancer

RESEARCH Open Access

Exosomal circLPAR1 functions in colorectal cancer diagnosis and tumorigenesis through suppressing *BRD4* via METTL3–eIF3h interaction

Rui Zheng[1,2†], Ke Zhang[3†], Shanyue Tan[3†], Fang Gao[2,4], Yajie Zhang[5,6], Wenxia Xu[7], Huabin Wang[7], Dongying Gu[8], Lingjun Zhu[3], Shuwei Li[1,2], Haiyan Chu[1,2], Zhengdong Zhang[1,2], Lingxiang Liu[3*], Mulong Du[1,9*] and Meilin Wang[1,2,10,11*]

Principal aims

Diagnostic evaluation and clinical perspective - identify and evaluate the diagnostic potential of exosomal circLPAR1 for colorectal cancer (CRC) diagnosis

Functional role in tumorigenesis - the role of circLPAR1 in suppressing the CRC tumor growth *in vitro* and *in vivo*

Elucidation for molecular mechanism - the way that circLPAR1 acts inside the cells

Key points of experimental results

1. Introduction
2. circRNAs identification in colorectal cancer
3. circLPAR1 characterization
4. circLPAR1 as biomarker
5. Influence of circLPAR1 on cellular phenotypes *in vitro*
6. circLPAR1 interactions
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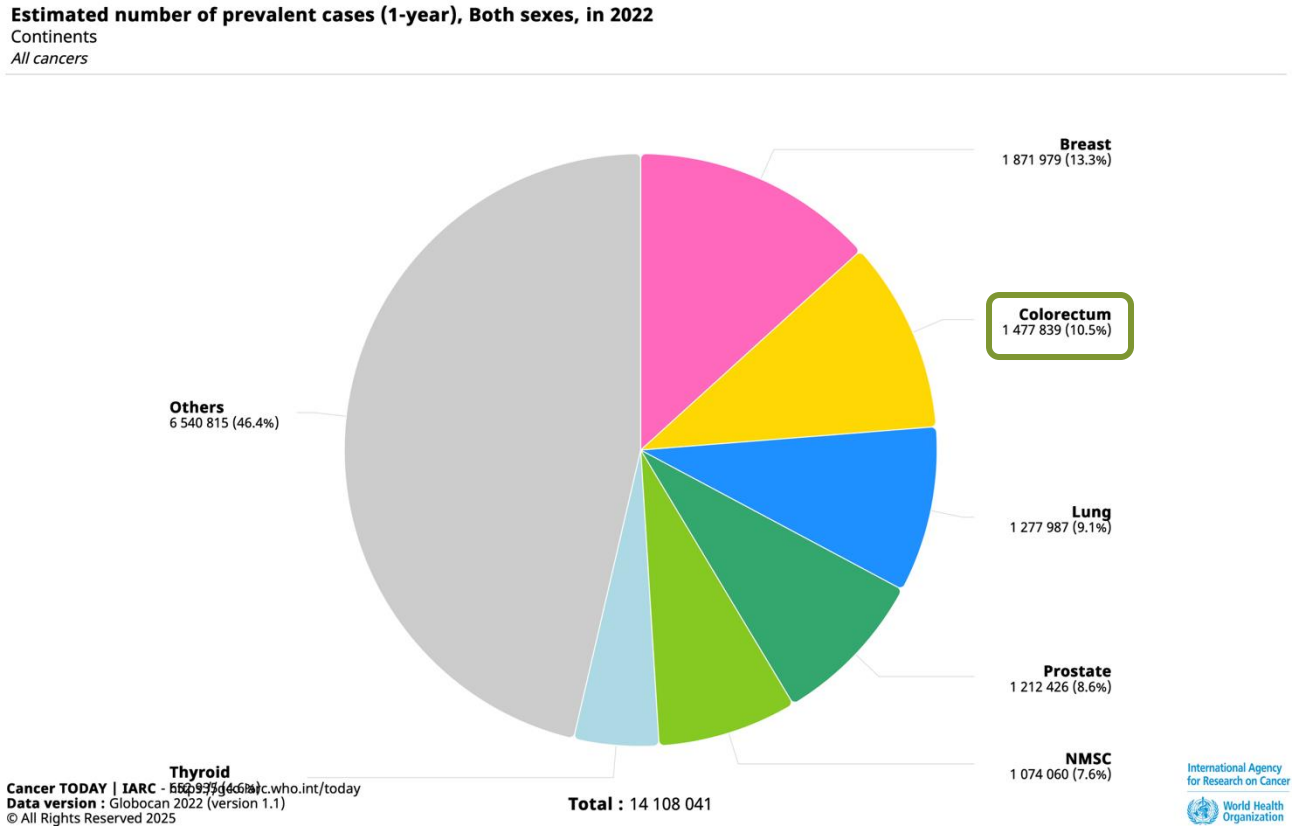
Colorectal cancer and circRNAs

Colorectal cancer (CRC):

- Is the **second leading cause** of cancer-related deaths worldwide
- 900.000 deaths in 2022
- 1.9 million new cases

Early detection and **diagnosis**
and
novel biomarkers



→ Exosomal circLPAR1 as a diagnostic tool and possible biomarker for colorectal cancer

Colorectal cancer and circRNAs

Samples that were considered:

- Cancer free controls
- Pre-cancer individuals
- Colorectal cancer patients + normal adjacent tissues (NATs)

Long-term follow-up clinical cohort

In every condition → corresponding tumour, normal tissues and peripheral blood were considered

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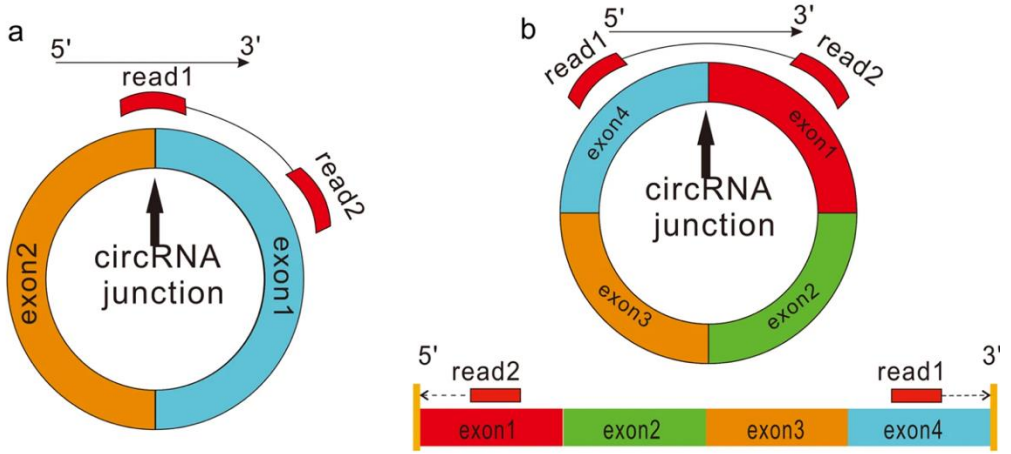
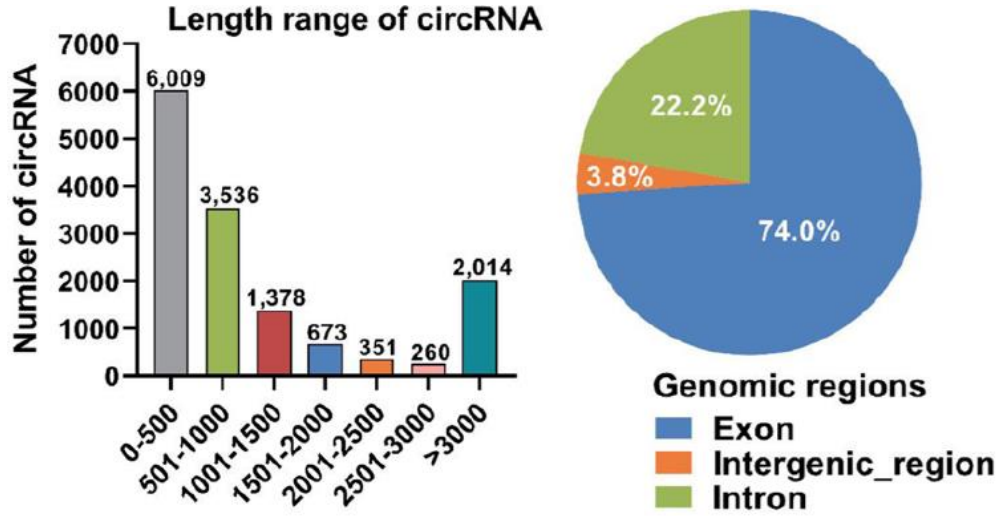
Candidate circRNAs identification

1. rRNA depletion and **total RNA Seq**: Tumour vs Normal Adjacent Tissues (NATs) (n=52)

2. *Find_Circ* and *CIRI2* algorithms: ***de novo* circRNAs identification** → based on Back-Splicing Junctions (**BSJ**) pairing



[Jia Gy. et al., 2019]



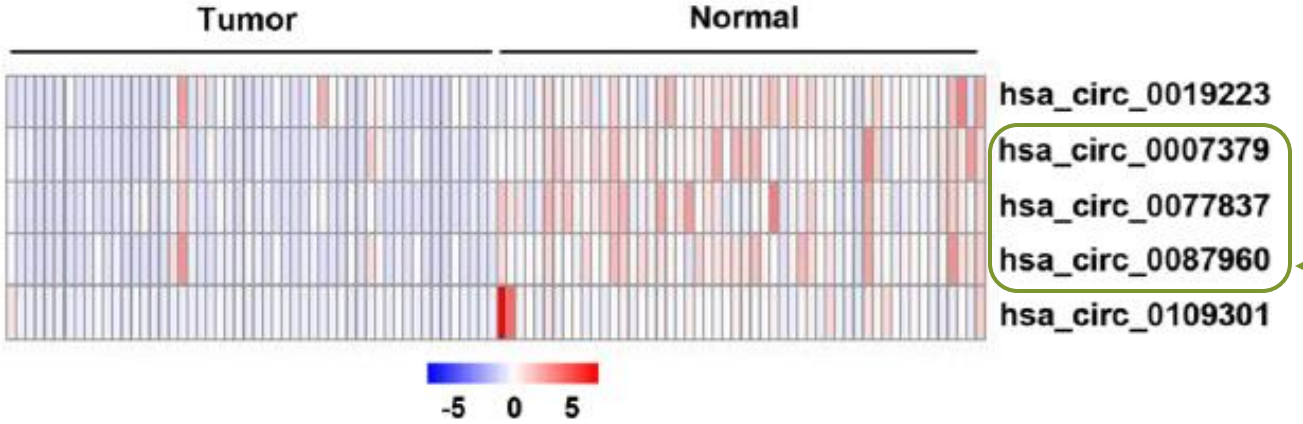
14 221 circRNAs identified

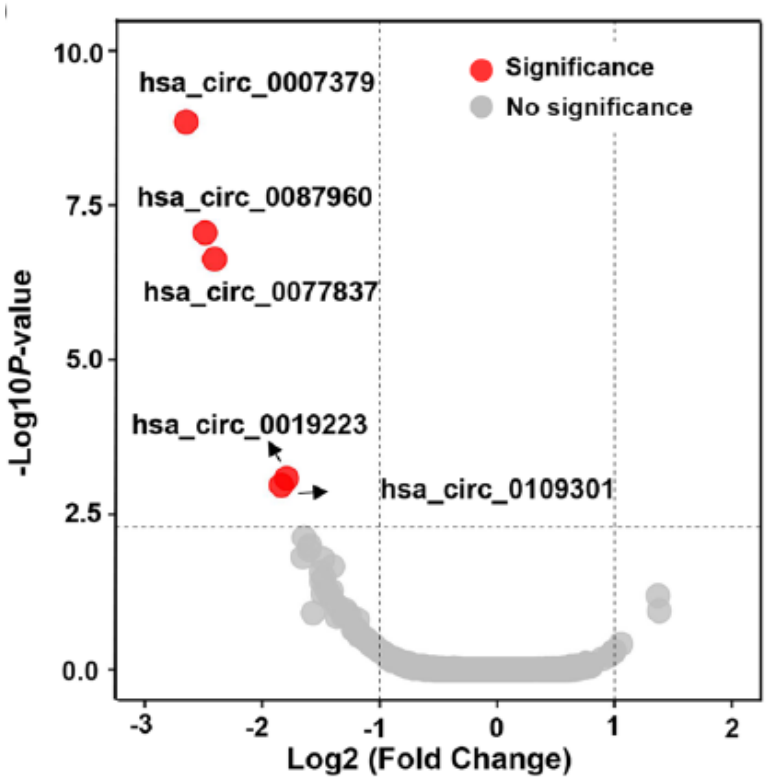
Downregulated circRNAs in CRC

3. circRNAs with *stable expression* discarded
4. Filter criteria: **fold change ≥|2|** and **P <0,005**
5. Focus on *downregulated* circRNAs

5 circRNAs significantly downregulated

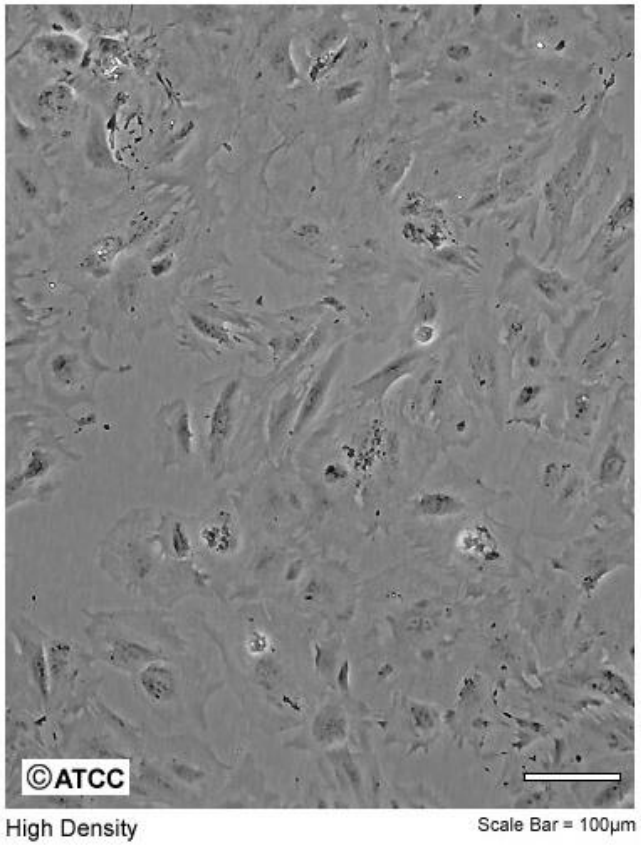
6. Annotation in **ExoRBase** → circRNAs present in exosomes



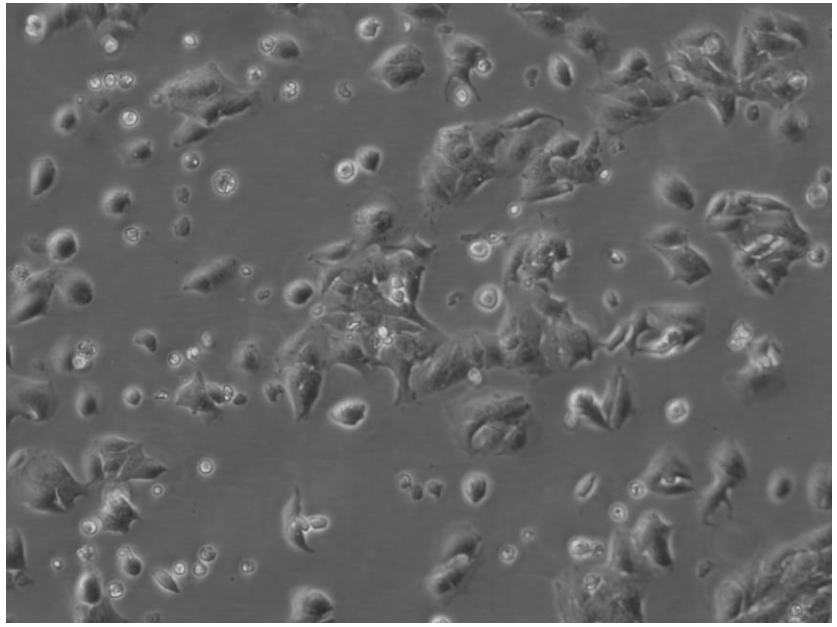


- *hsa_circ_0087960* (circLPAR1) identified among the exosomal circRNAs

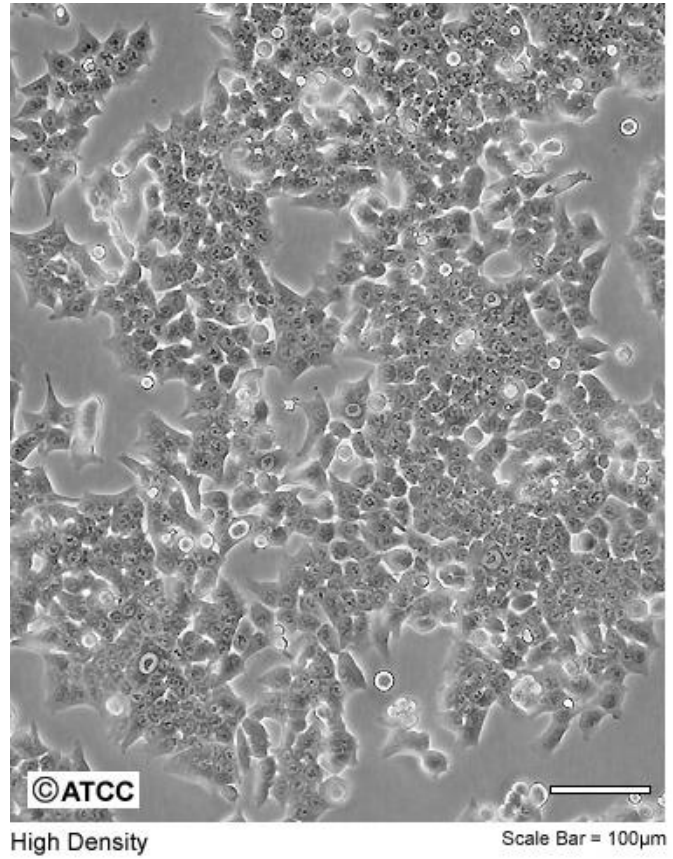
Cell line validation



FHC: human adherent cell line from the large intestine of a ***healthy*** donor



DLD1: human ***colorectal adenocarcinoma*** cell line from large intestine ***MSH6*** **deficient**



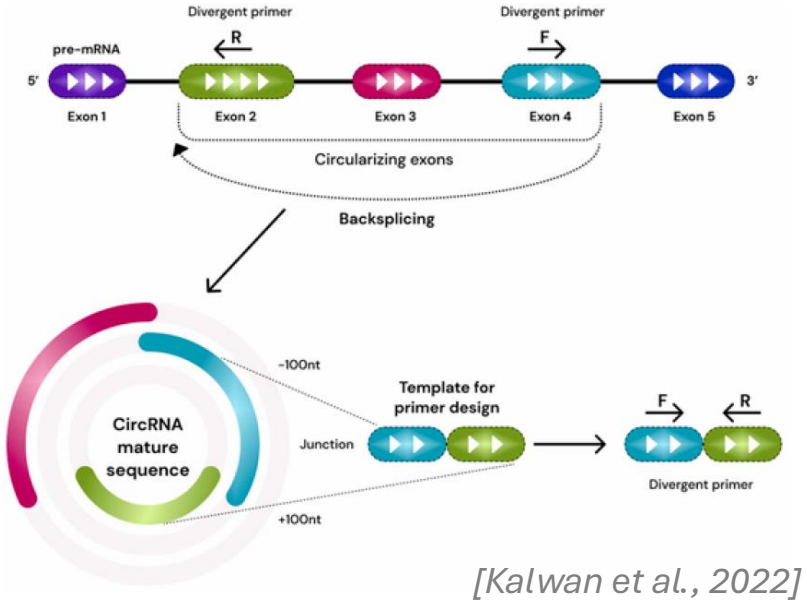
HCT-116: adherent cell line from ***colon cancer, MLH1/MSH3*** **deficient**

Exosomal circLPAR1 as a candidate

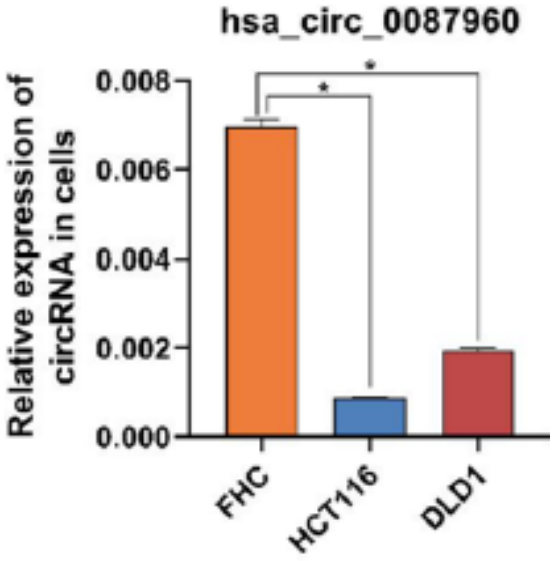
Cell line validation: CRC (HCT116 and DLD1) vs healthy cells (FHC)

1. RNAse R digestion of linear mRNAs → circularity confirmed
2. RT-qPCR with ***divergent primers*** → differential expression in cells

CircLPAR1 is the only one **significantly downregulated** in CRC cells

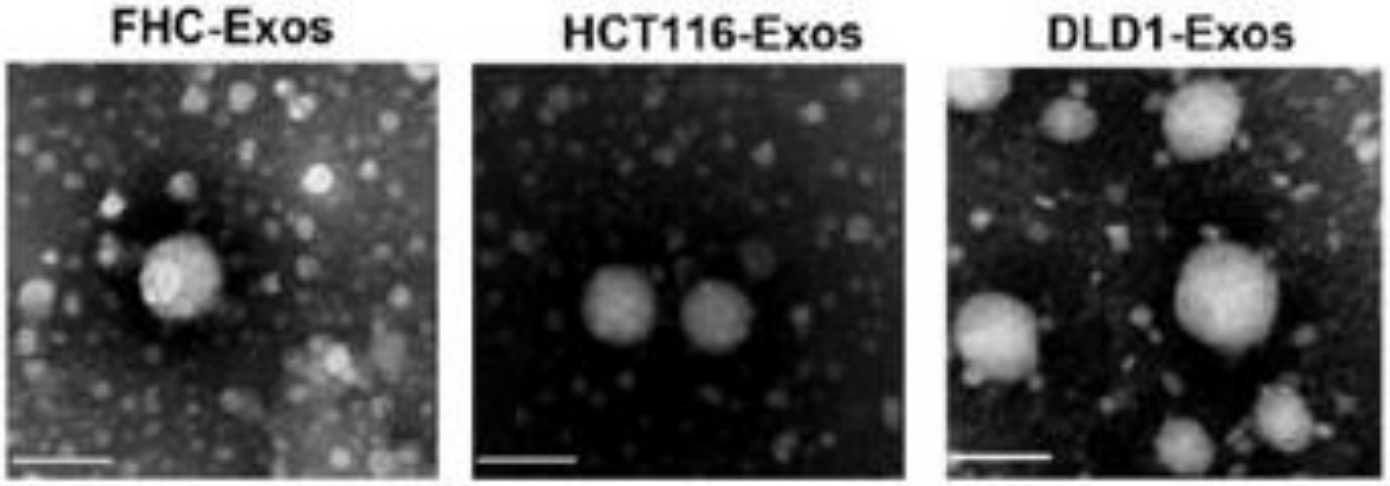


[Kalwan et al., 2022]

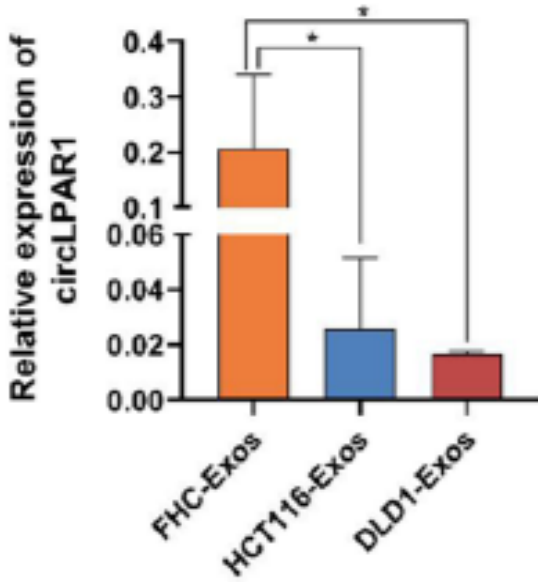


Normalized to GADPH cDNA

Exosomes purification



Scale bar 100 nm



Normalized to GADPH cDNA

1. Exosome isolation from culture medium → centrifugation at low speeds and purification
2. Nanoflow cytometry and TEM → exosome size and shape characterization
3. Western Blot → exosome-specific markers **Alix** and **TSG101**
4. RT-qPCR → circLPAR1 relative expression
5. *Exosome secretion inhibitor GW4869* → less circLPAR1 in culture medium

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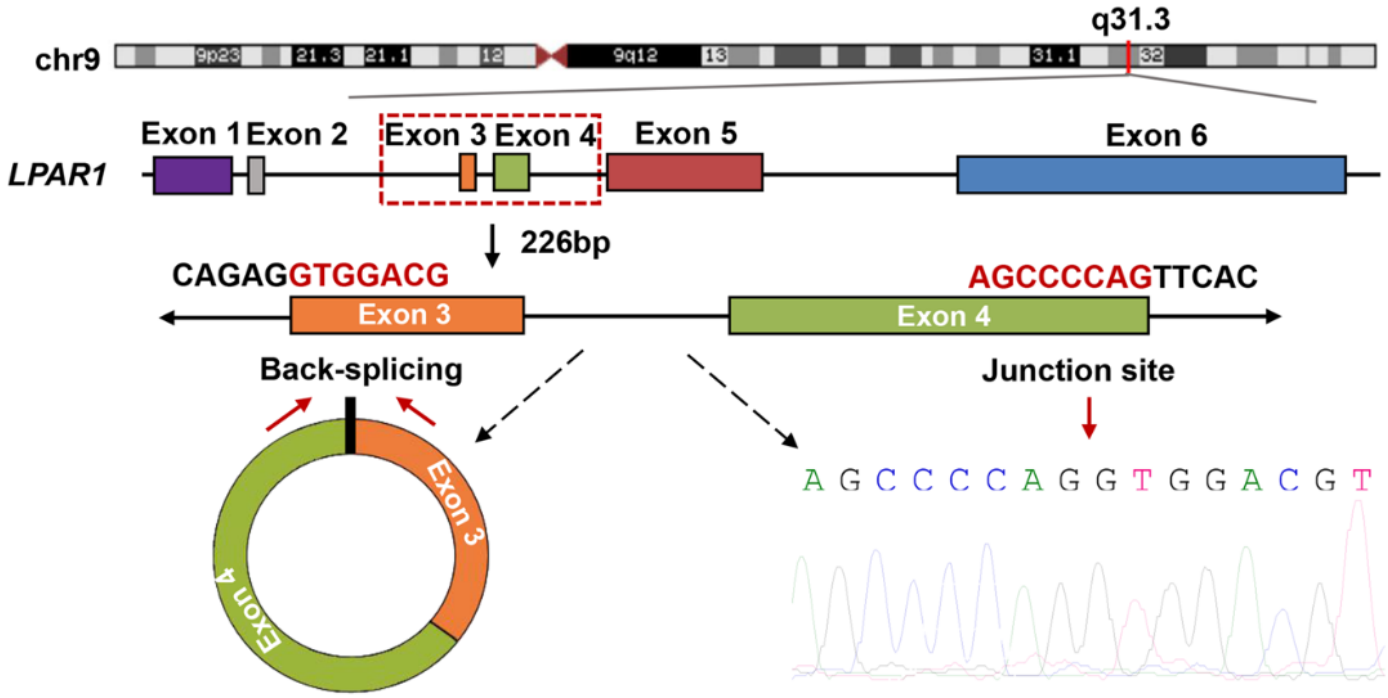
CircLPAR1 characteristics

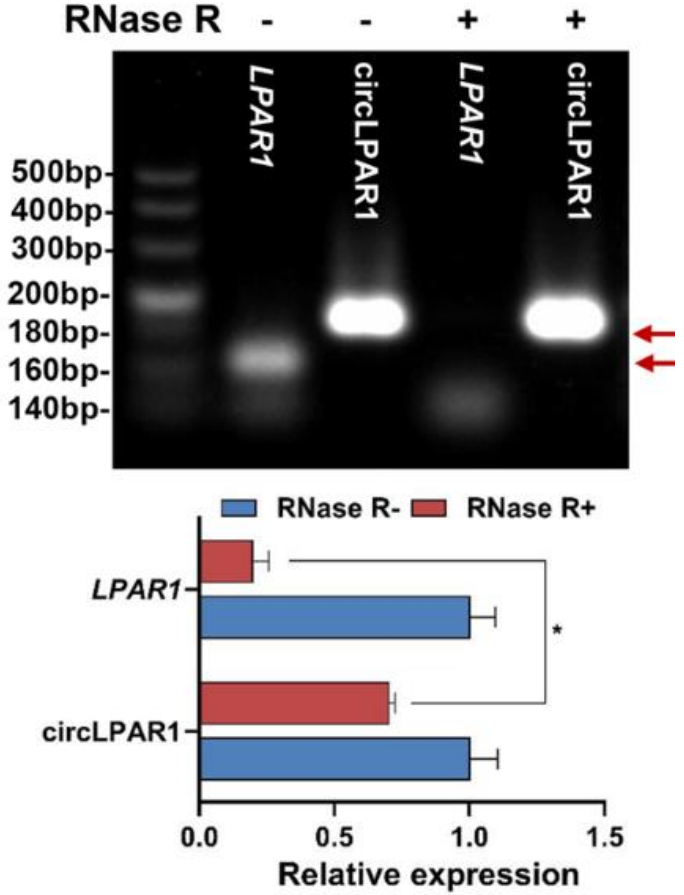
circLPAR1: 226 bases long with head to tail back splice junction site, circularization of **exon 3** and **exon 4** of the LPAR1 gene (chr9)



CircLPAR1 characteristics

Stability: circLPAR1 is more stable than the linear transcript → treatment with actinomycin D

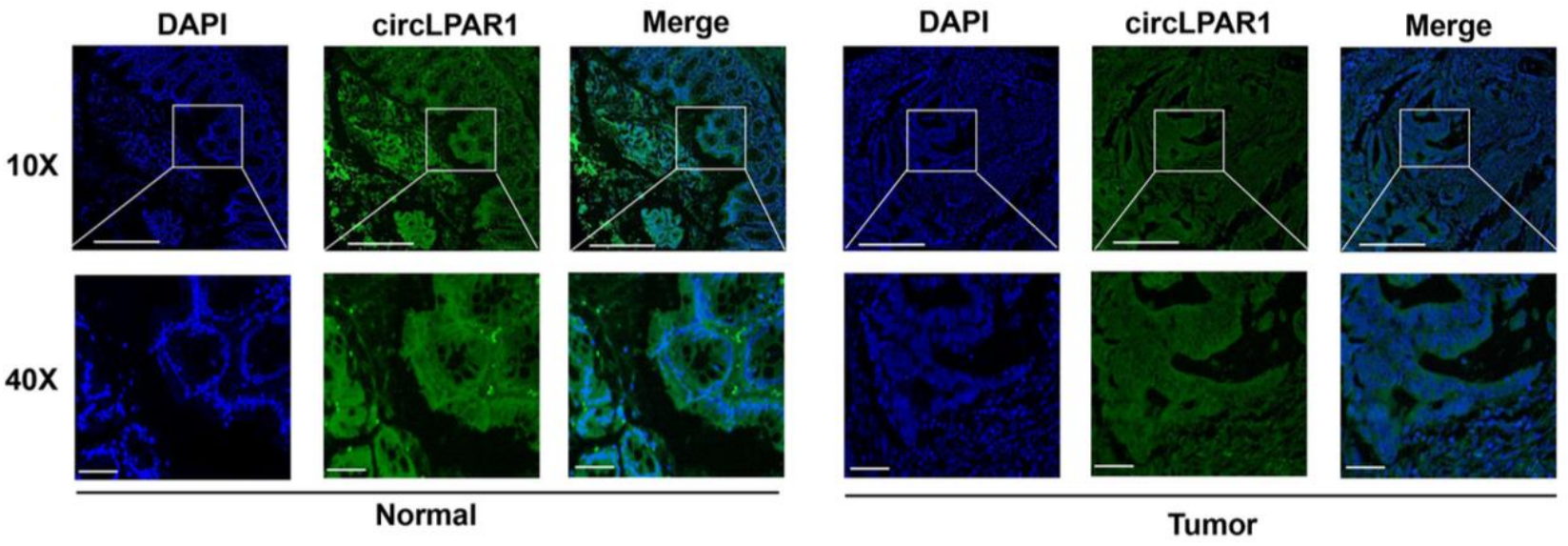
Resistance to digestion: circLPAR1 resistant to RNase R digestion compared to the linear transcript

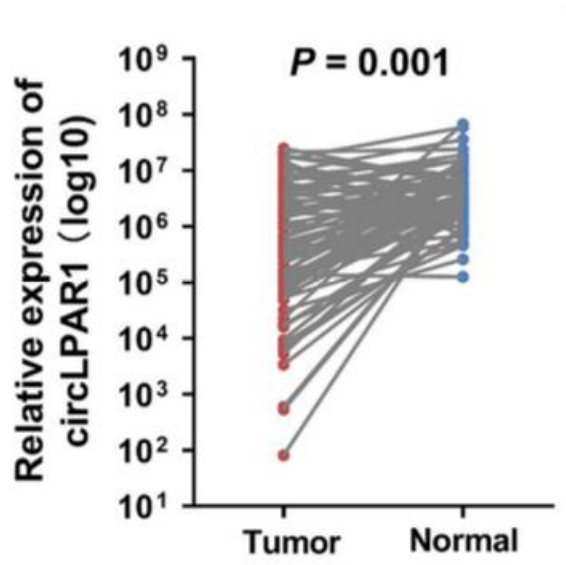


CircLPAR1 characteristics

FISH assay using colorectal and NATs samples → circLPAR1 is expressed at lower levels in colorectal tumor cells than in NATs

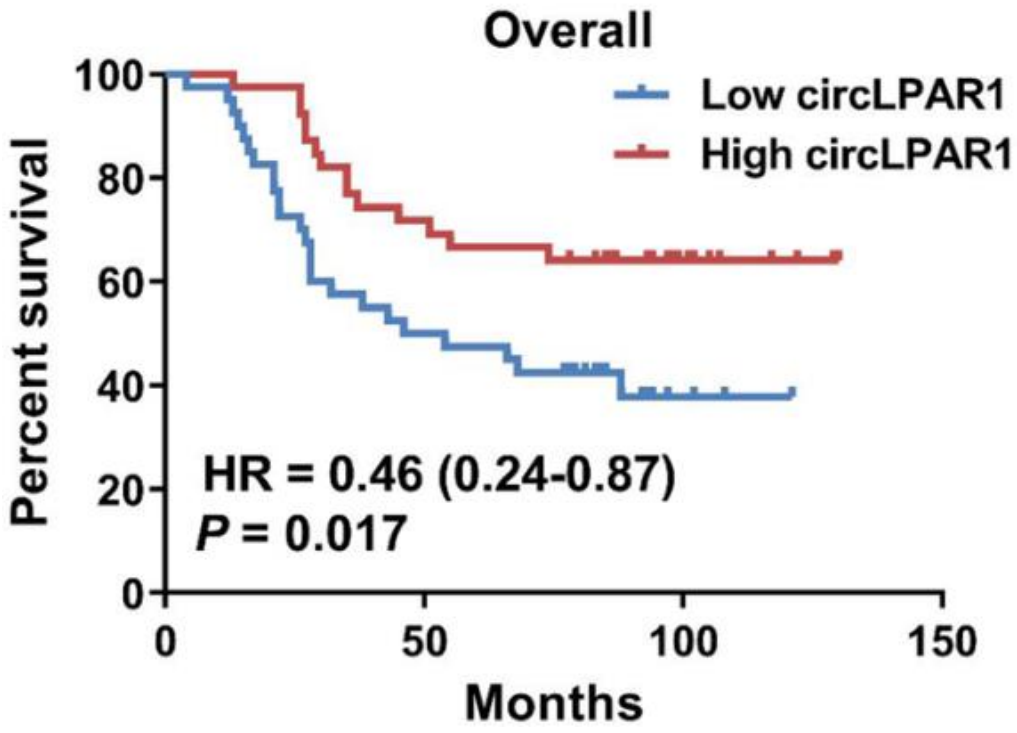
→ circLPAR1 is present in the **cytoplasm**





CircLPAR1 characteristics

→ Colorectal cancer patients with **high circLPAR1 level** exhibited significantly better overall **survival** than those with low circLPAR1 level



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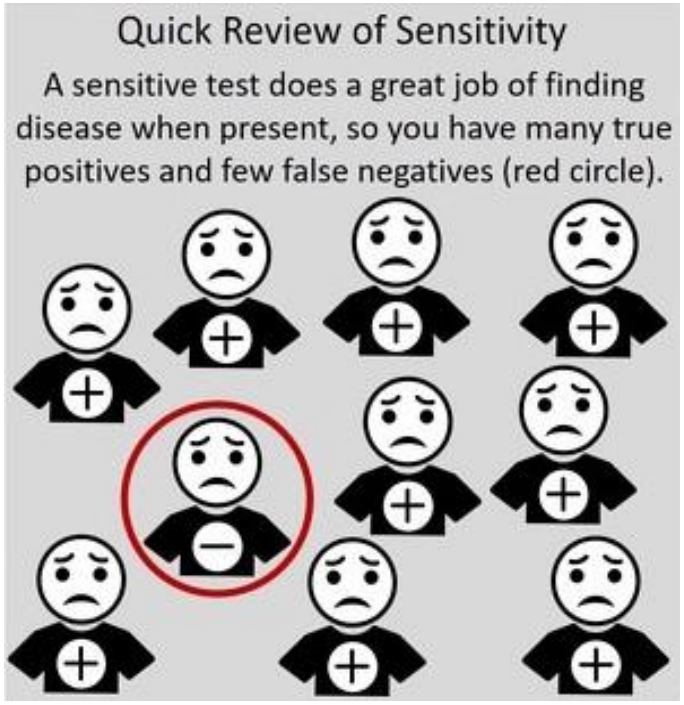
How to assess circLPAR1 diagnostic potential?

Specificity

Ability to distinguish ALL healthy individuals

Sensitivity

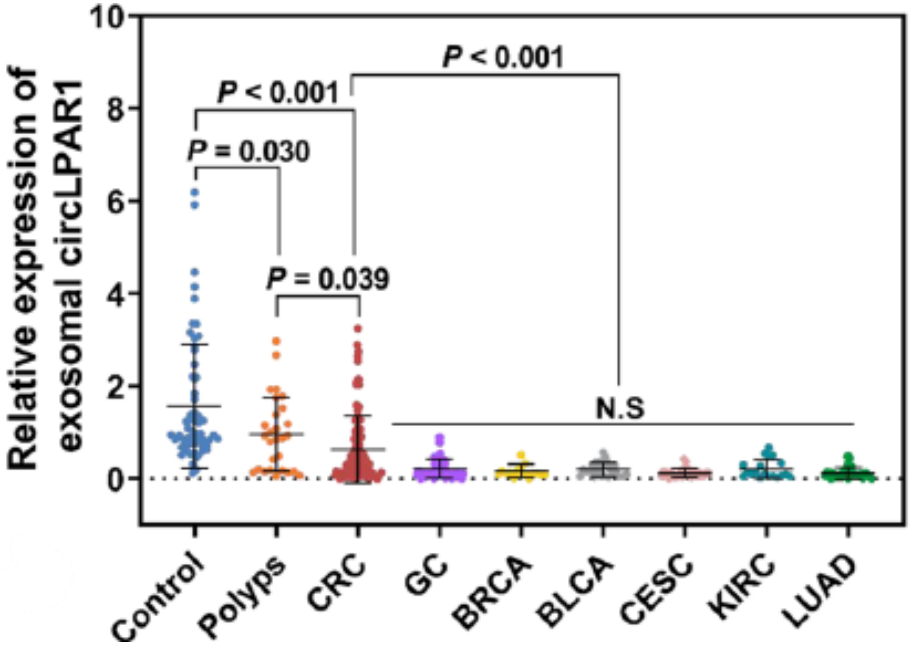
Ability to find ALL patients with cancer

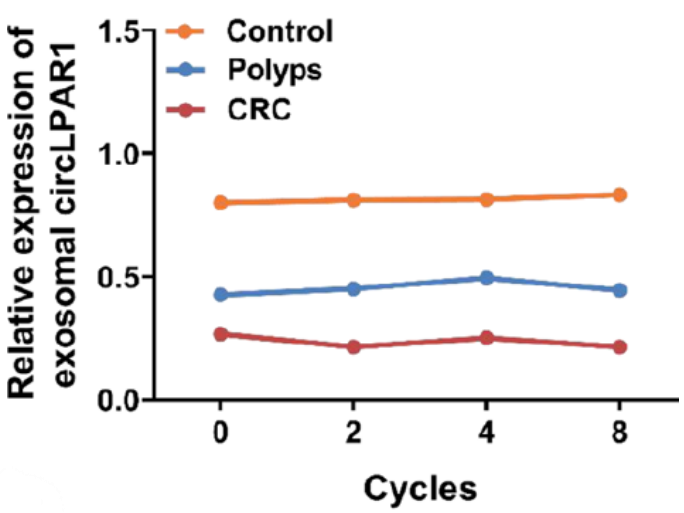


[Tigges S., 2021]

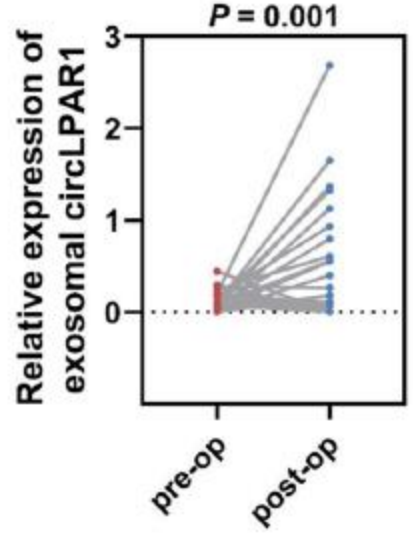
CircLPAR1 as CRC biomarker?

- ✓Highly **stable**
- ✓*Decreasing* levels: Healthy>Polyps>CRC
- ✓*Increasing* post tumour resection
- ✓Found in exosomes → **easily accessible**





Freeze and thaw cycles

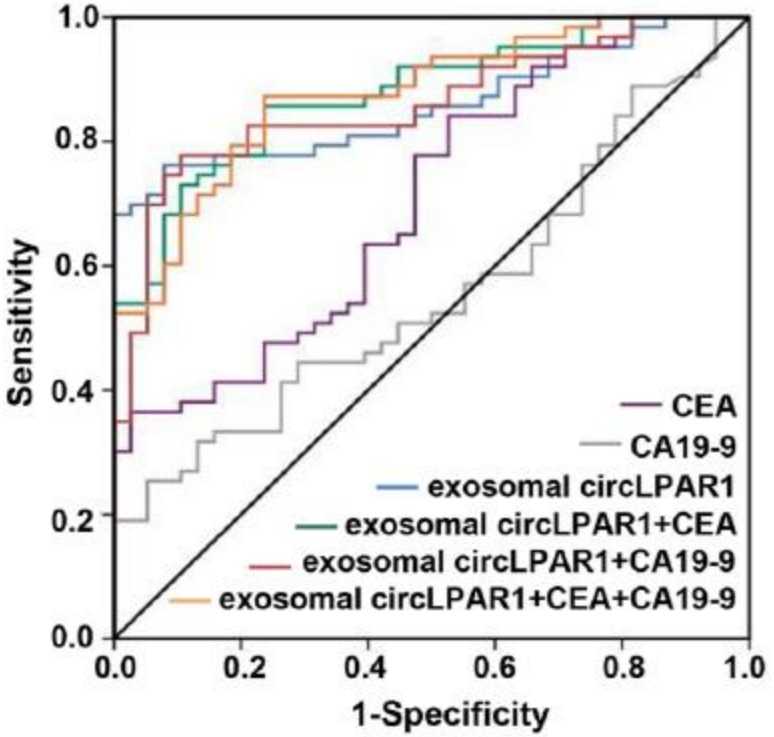


- ✓**Specific** for CRC

CircLPAR1 levels in patients with other cancer types *significantly* different and lower than CRC ones

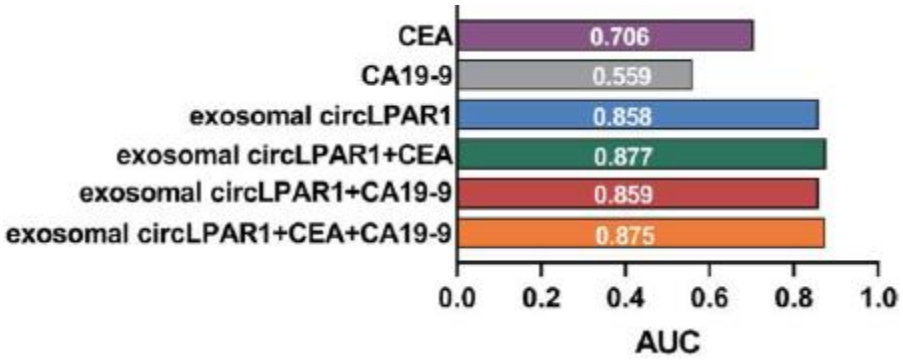
CircLPAR1 vs adopted CRC biomarkers

- *Receiver Operating Characteristics* (**ROC**) curve → **diagnostic marker performance**
- **AUC**= *Area Under the Curve* → **accuracy** of a diagnostic test
- ***CEA*** and ***CA19-9*** widely used for CRC diagnosis
- Exosomal ***circLPAR1 AUC > CEA/C19-9 AUC***



circLPAR1 *alone or in combination* is a **specific CRC diagnostic biomarker**

SPECIFICITY: 76,30%
SENSITIVITY: 87,30%



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2. circRNAs identification in colorectal cancer
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6. circLPAR1 interactions
7. Influence of circLPAR1 on cellular phenotypes *in vivo*

CircLPAR1 influence on tumor cell phenotype

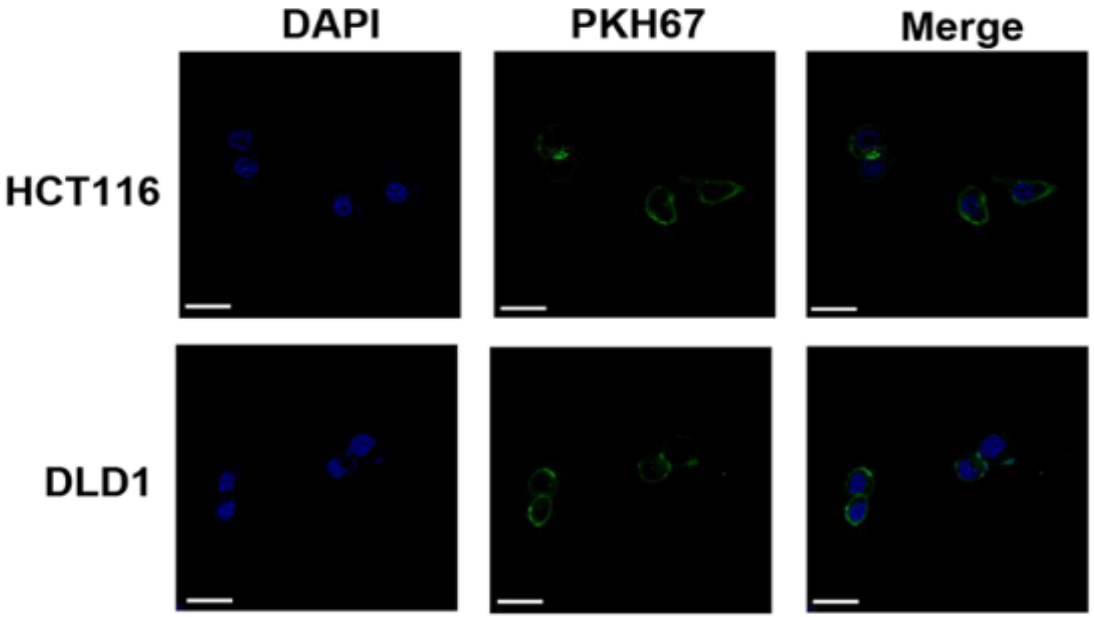
Exploration of biological function of exosomal circLPAR1 in colorectal tumorigenesis

Fluorescent microscopy with PKH67 fluorescent dye:

- Group treated with exosomes – presence of the dye in the **cytoplasm** of cells
- Group that is not treated with exosomes – no dye present in the cytoplasm

→ Exosomes are rapidly **adsorbed by tumor cells**

Addition of cytochalasin D → elimination of the uptake process



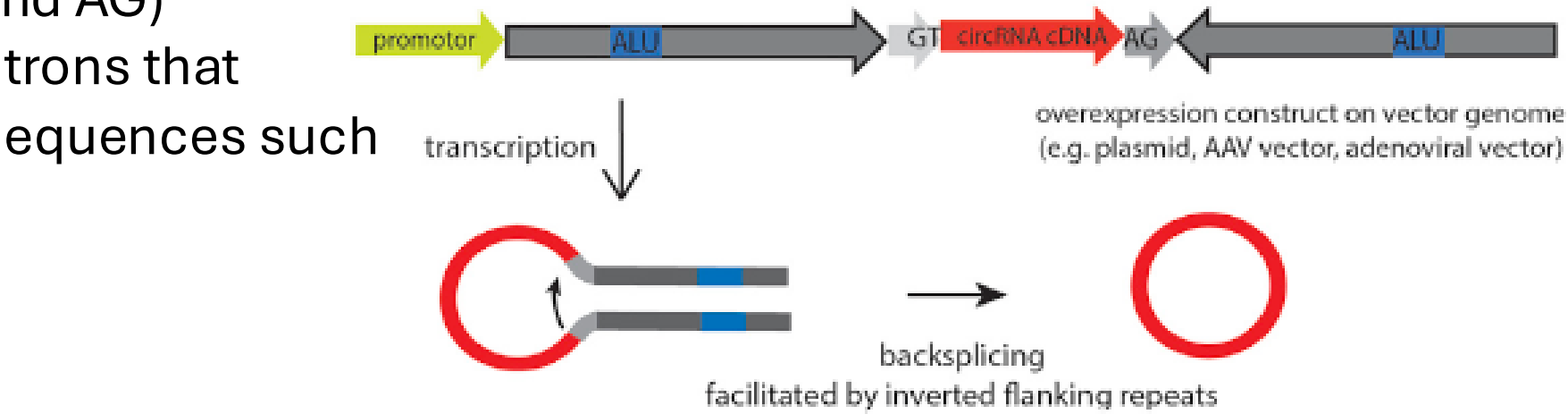
Scale bar 25 µm

CircLPAR1 influence on tumor cell phenotype

circLPAR1 gene cloned in **pLC5-circR expression vector** and pLC5 vector was used as the control (non-coding vector)

Elements in the vector for circular RNAs production:

- **Sequence of circLPAR1** (exon 3 and 4)
- Splice sites recognized by the spliceosome (GT and AG)
- Complementary introns that contain repeated sequences such as Alu



[Ubigene]

CircLPAR1 influence on tumor cell phenotype

Transfection of colorectal cancer cells:

- **circLPAR1-Exos** – HCT116 and DLD1 transfected with vector that encodes for circLPAR1
- **NC-Exos** – HCT116 and DLD1 transfected with non-coding vector

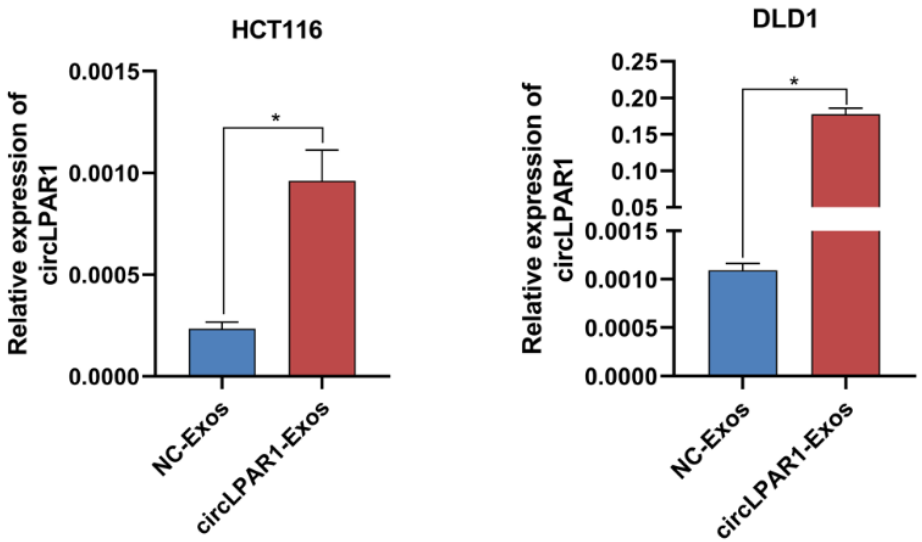
→ The exosomes produced by these cells are isolated

HCT116 and DLD1 incubated with these exosomes

→ **increase of circLPAR1 levels** in circLPAR1-Exos group

CircLPAR1 influence on tumor cell phenotype

→ **increase of circLPAR1 levels** in circLPAR1-Exos group

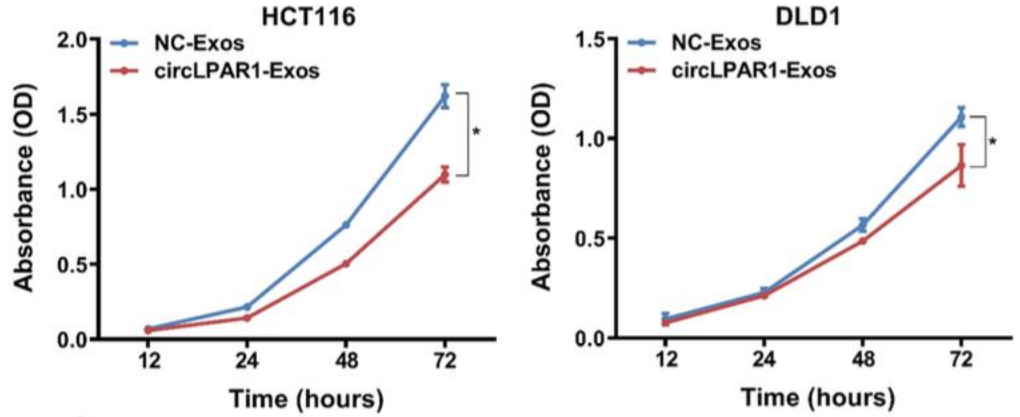


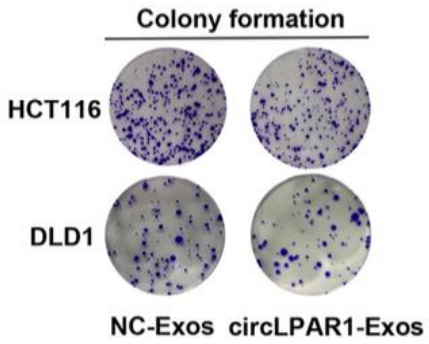
Suppression of proliferation, colony formation, invasion and migration of colorectal cancer cells

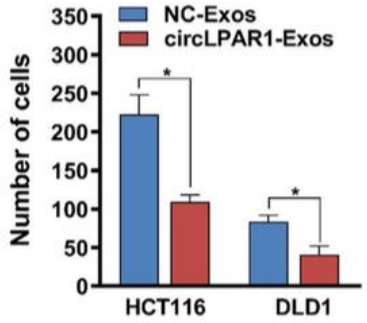
CircLPAR1 influence on tumor cell phenotype

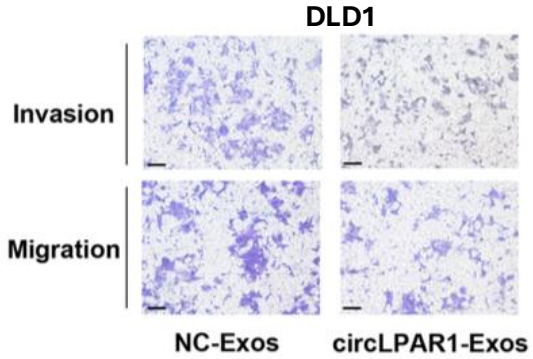
→ Suppression of proliferation, colony formation, invasion and migration of colorectal cancer cells

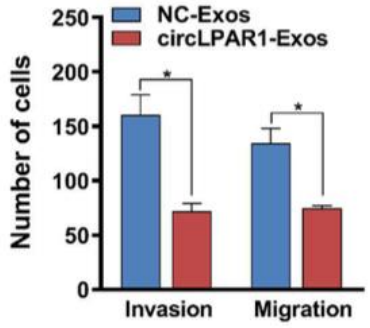
- Proliferation – CCK-8 assay
- Colony formation – colony formation assay
- Invasion and migration – transwell assay







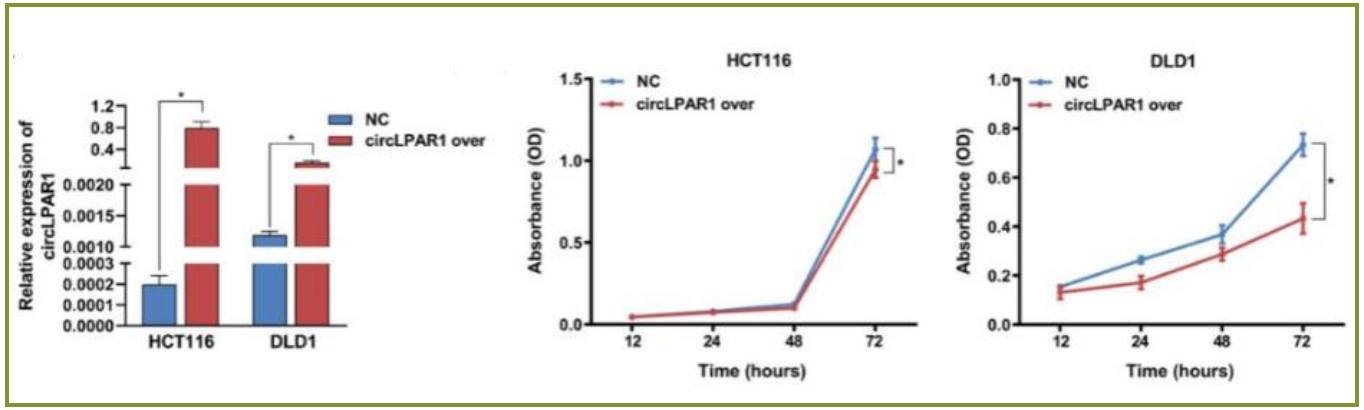




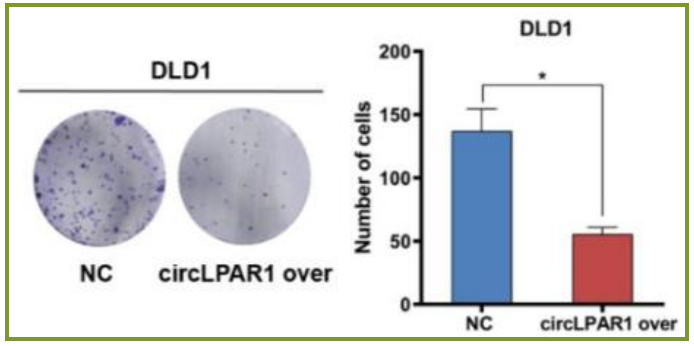
CircLPAR1 influence on tumor cell phenotype

→ Same result by direct overexpression of circLPAR1

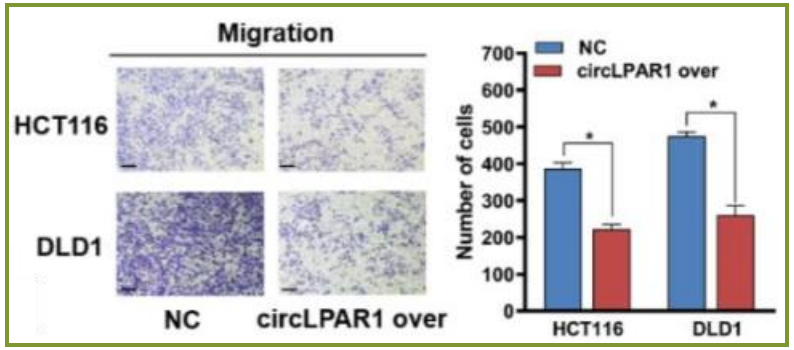
Proliferation



Colony formation



Migration



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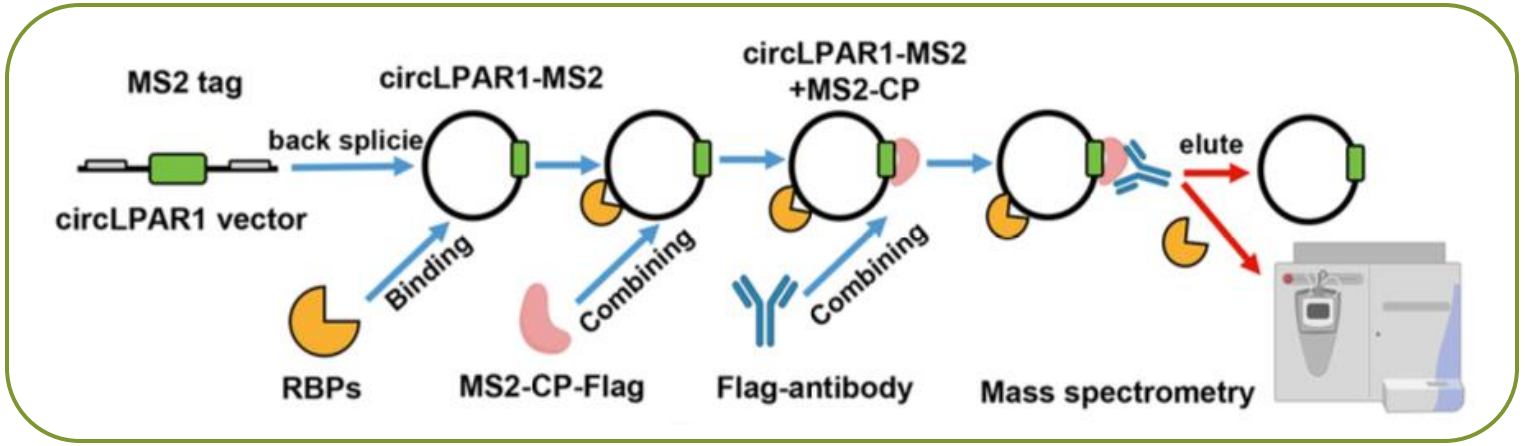
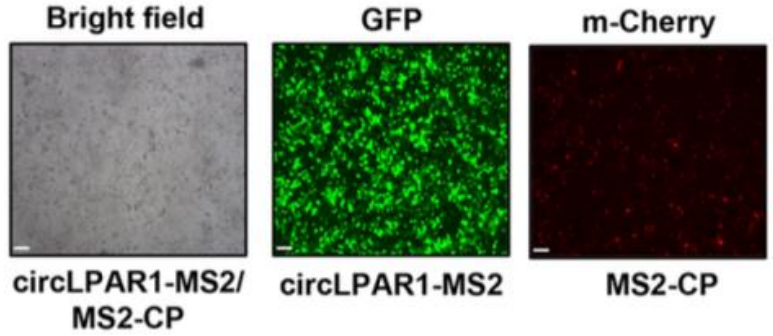
CircLPAR1 and RBPs interaction



Pull down assay:

- Co-transfection of HCT116 – two vectors used **circLPAR1-MS2** and **MS2-CP-flag**, respectively labelled with green and red fluorescent proteins
- Pull down assay with anti-flag antibody
- Control of the results – RT-qPCR and WB to confirm the captured products
- **Mass spectrometry** – to identify the RBPs that interact with circLPAR1



Scale bar 50 μm

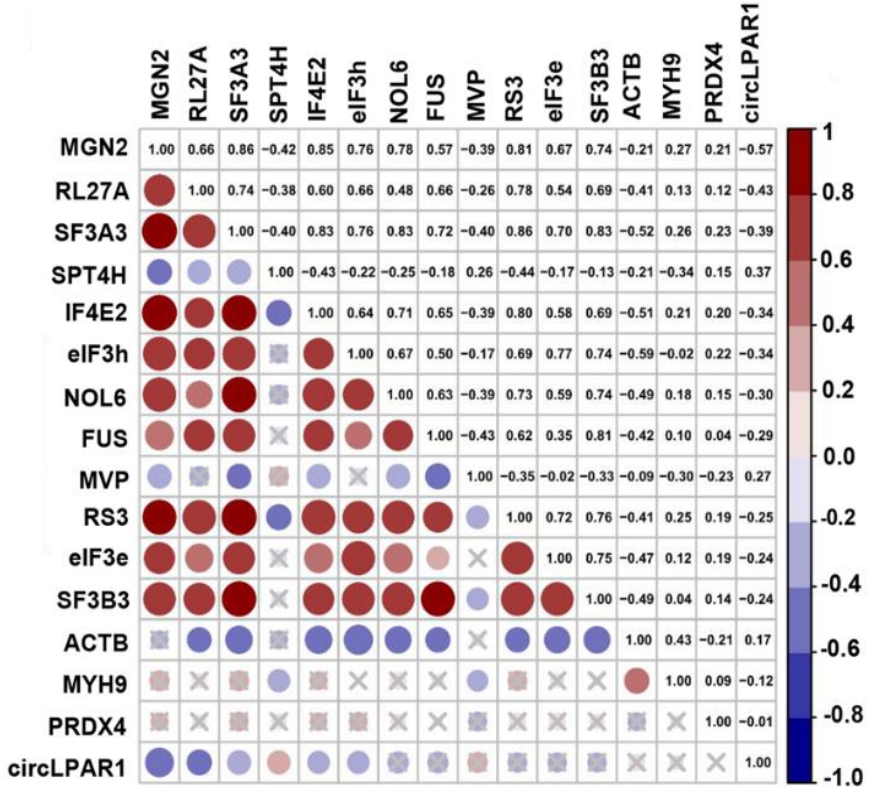
CircLPAR1 and RBPs interaction

- **Mass spectrometry** → 23 RBPs candidate interact with circLPAR1
- Differential expression analysis of these RBPs in colorectal tumors and NATs

  → 12 are upregulated, 3 are downregulated in tumor samples
- 6 candidates are selected based on Spearman's statistical correlation (P<0,05)

Binding capacity of the candidates → catRAPID

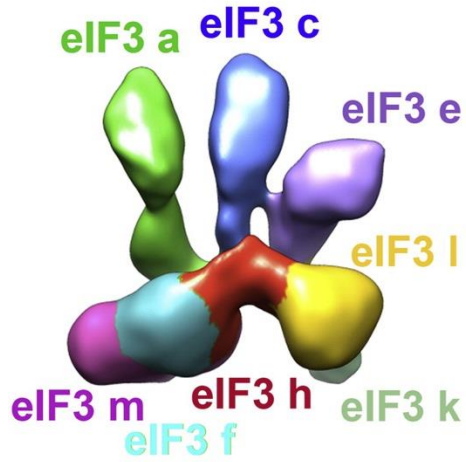
eIF3h has the strongest binding capacity with circLPAR1

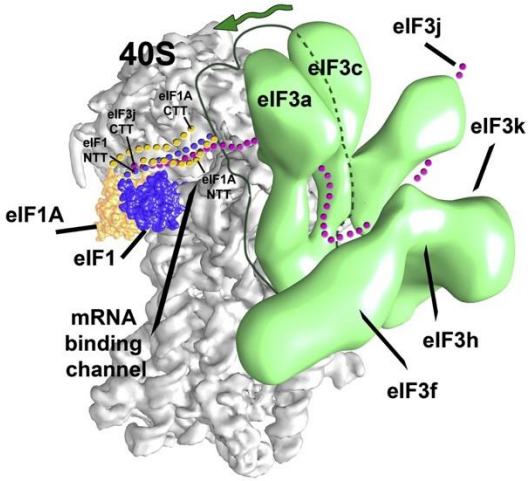


CircLPAR1 and eIF3h

eIF3h – eukaryotic translation Initiation Factor 3 subunit h → is part of eIF complex involved in translation

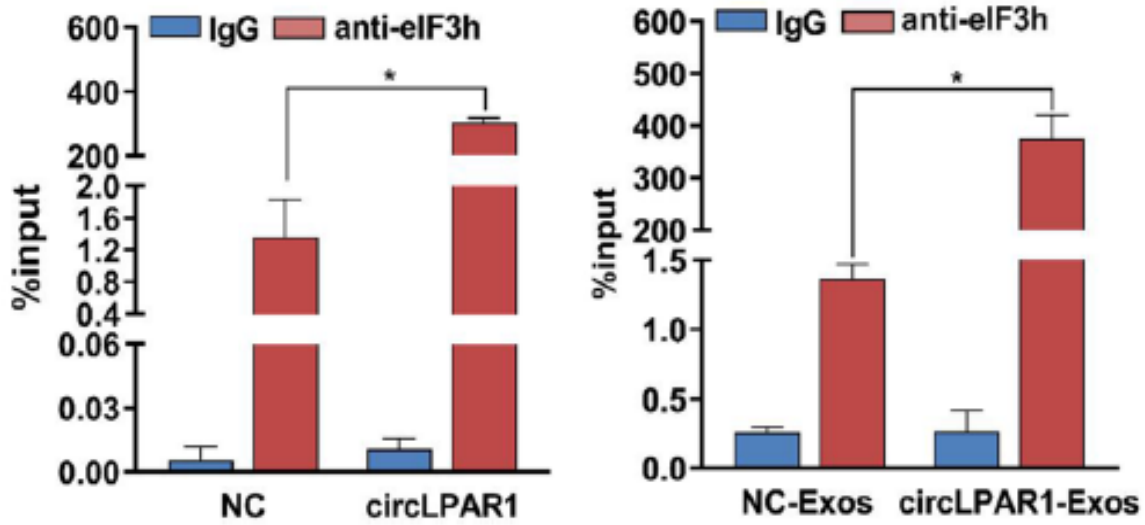
- The eIF-3 complex associates with the 40S ribosome and facilitates the recruitment of other factors to form the **pre-initiation complex**
- The eIF-3 complex specifically targets and initiates translation of a subset of mRNAs involved in **cell proliferation**, including cell cycling, **differentiation** and **apoptosis**

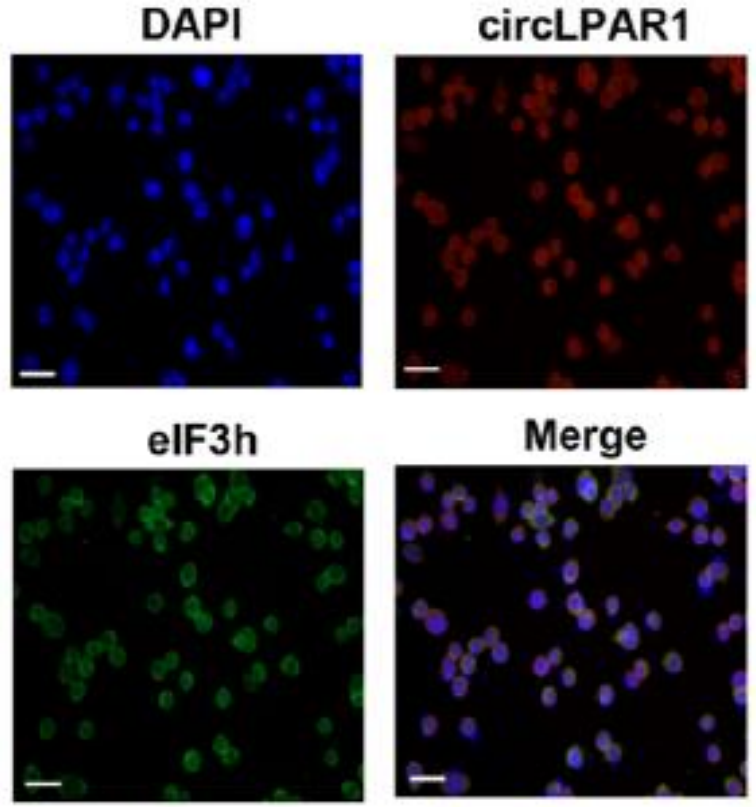




CircLPAR1 – eIF3h interaction

- *IF*: **Colocalization** eIF3h-circLPAR1 in cytoplasm (DLD1 cells)
- *RIP assay*: control (NC or NC Exos) vs CRC cells 1.transfected with circLPAR1 or 2. exposed to circLPAR1 exosomes



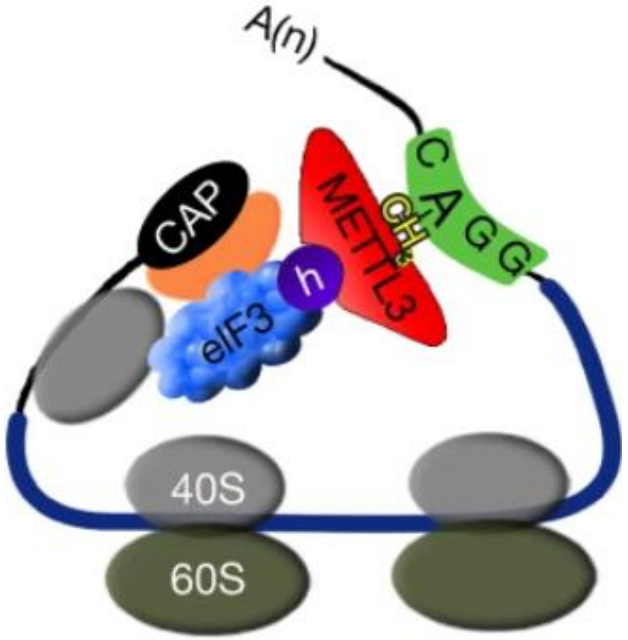


Scale bar 50 μm

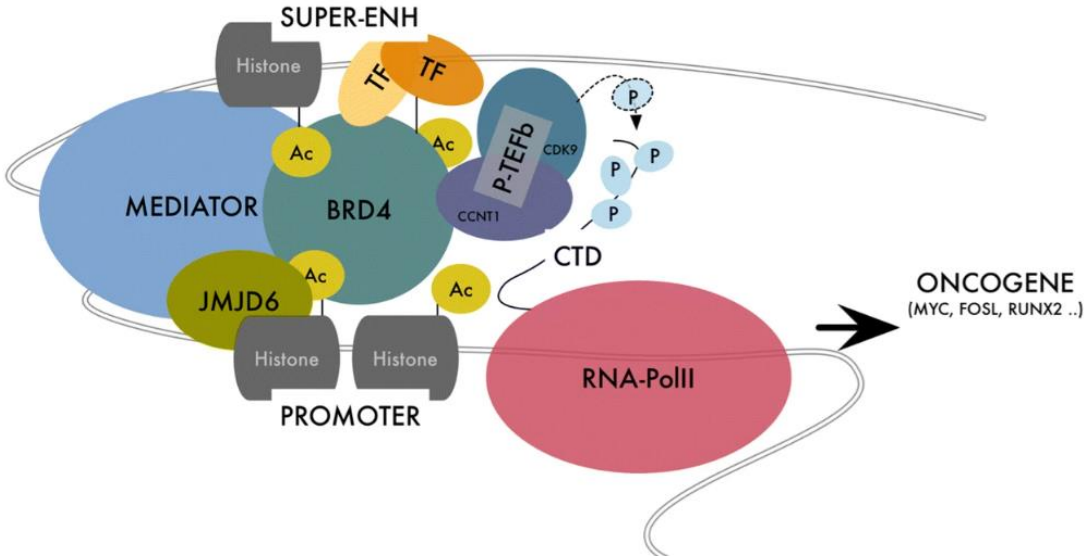
CircLPAR1-eIF3h physical association

From previous studies…

- Lung cancer: eIF3h shown to *directly interact* with METTL3 → BRD4 mRNA stabilization
- *METTL3*: **m^{6}A** writer, acts on 3' UTR of mostly oncogenic mRNAs
- mRNA looping →**enhanced translation**



[Choe et al., 2018]

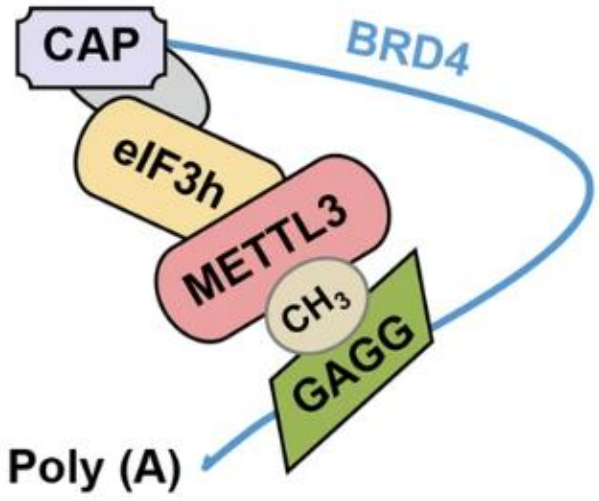


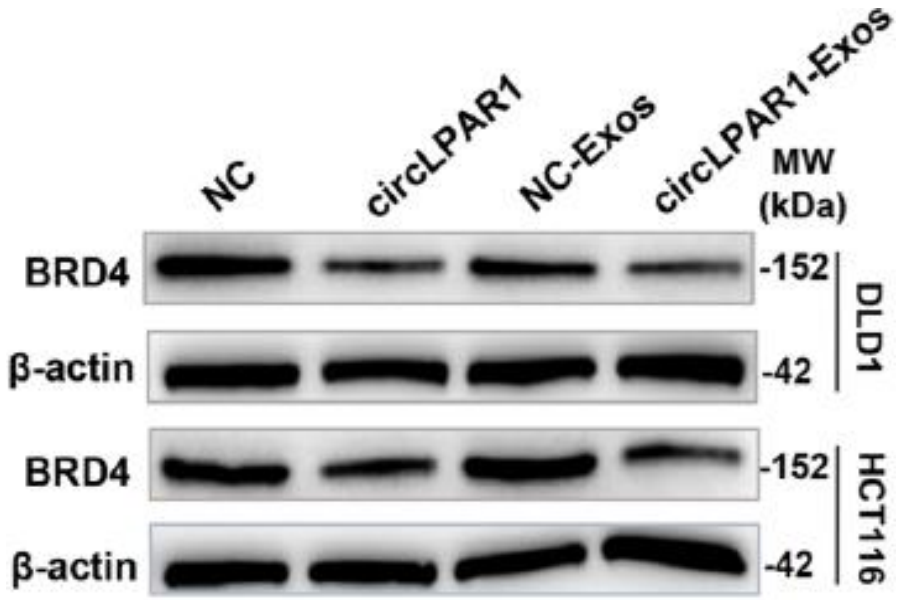
[Donati et al., 2018]

- ***BRD4*** → histone acetylation reader
- Productive elongation: *pTEFb-CDK9* recruitment
- *Super enhancers* (***H3K27Ac***): association with Mediator Complex → ex: *Myc* transcription

Is circLPAR1 a sponge for eIF3h?

Hypothesis: circLPAR1 as a **sponge for eIF3h** → influence on BRD4 levels?



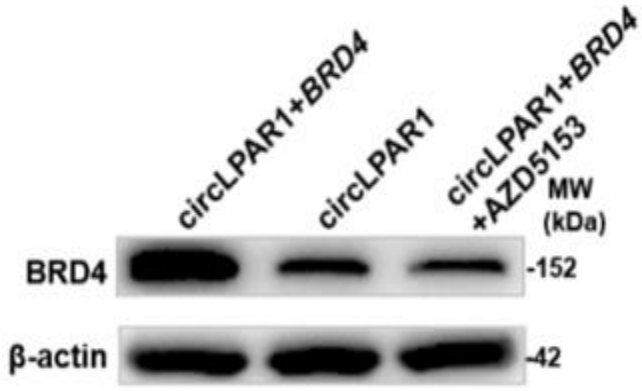


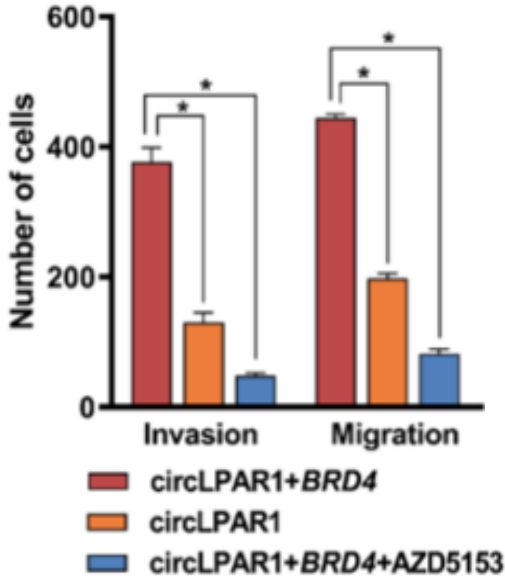
- Cells 1. overexpressing circLPAR1 or 2. incubated with circLPAR1 exosomes
- Control (NC and NC-Exos) vs treated
- WB: circLPAR1-treated cells show **decreased level of BRD4**

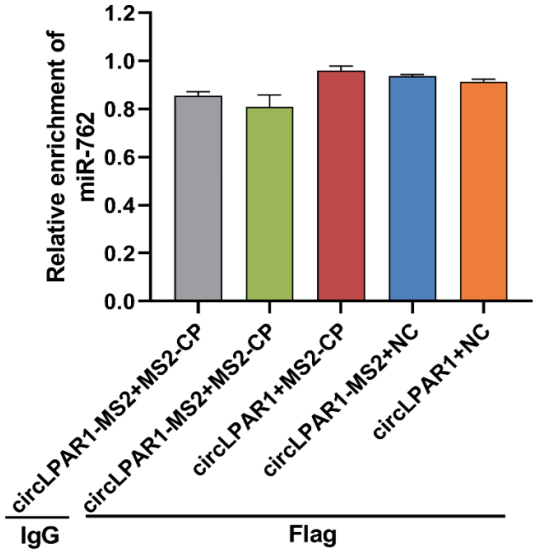
Is BRD4 the downstream functional effector?

Rescue experiment:

- **BRD4 overexpression** in CIRCLPAR1-expressing cells → aggressive cancer phenotype
- CircLPAR1 cells → reversal in tumoral behaviour
- Positive control: ***BRD4 inhibitor*** AZD5153







- Bladder cancer: circLPAR1 sponging ***miR-762*** ← → ***migration*** *and metastasis*
- *CircRNA pulldown*: **no enrichment** in miR-762

BRD4 is the critical protein for circLPAR1 cellular function

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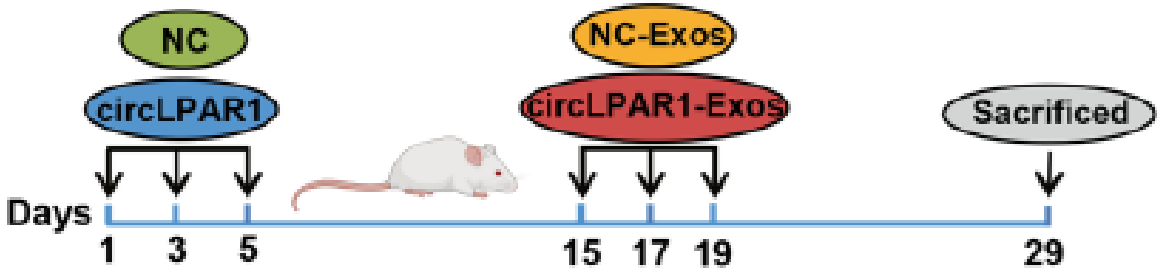
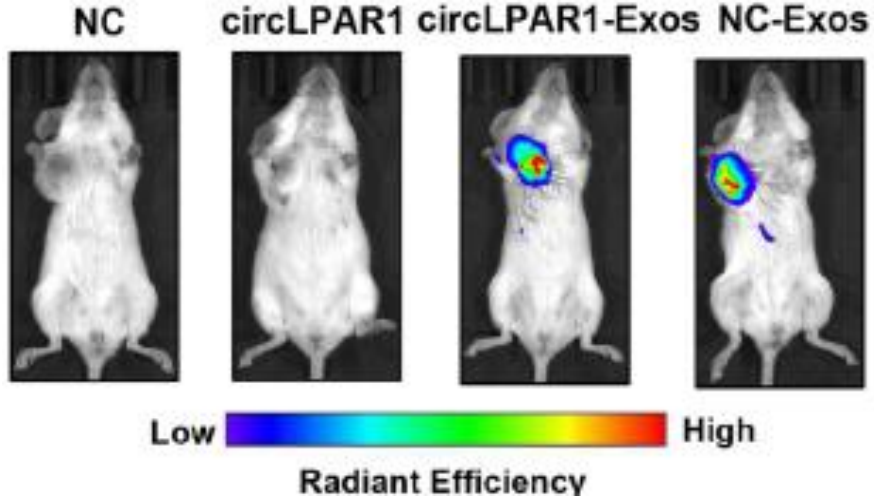
What are circLPAR1 effects *in vivo*?

- Triple immunodeficient ***NCG*** mice
- Function of ***intracellular*** *circLPAR1* → *Day 1, 3 and 5*: control or stably overexpressing circLPAR1 DLD-1 **cells injected** in mice
- Effect of ***exosomal*** circLPAR1 → *Day 15, 17, 19* : intratumoral **injection of stained exosomes** derived from control or circLPAR1-overexpressing DLD1 cells
- Tumour width and lenght measured once every 2 days

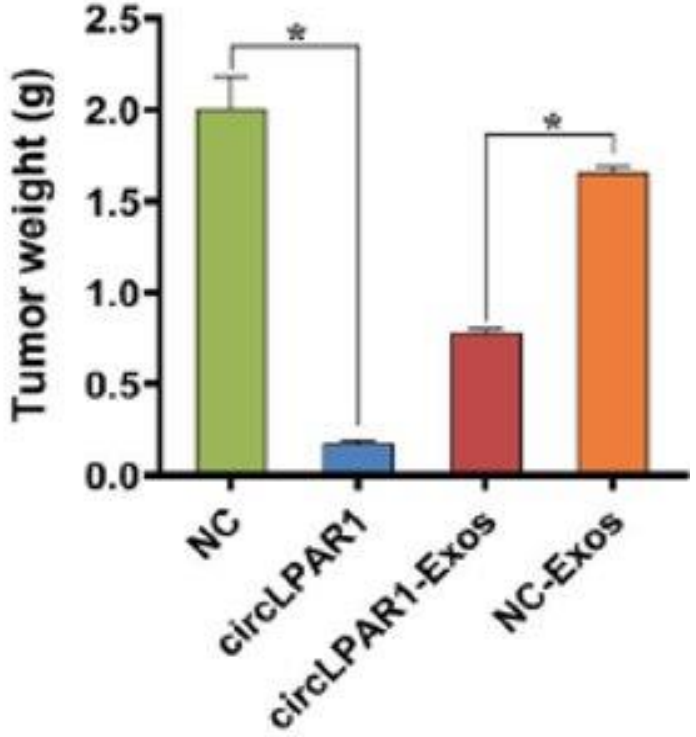




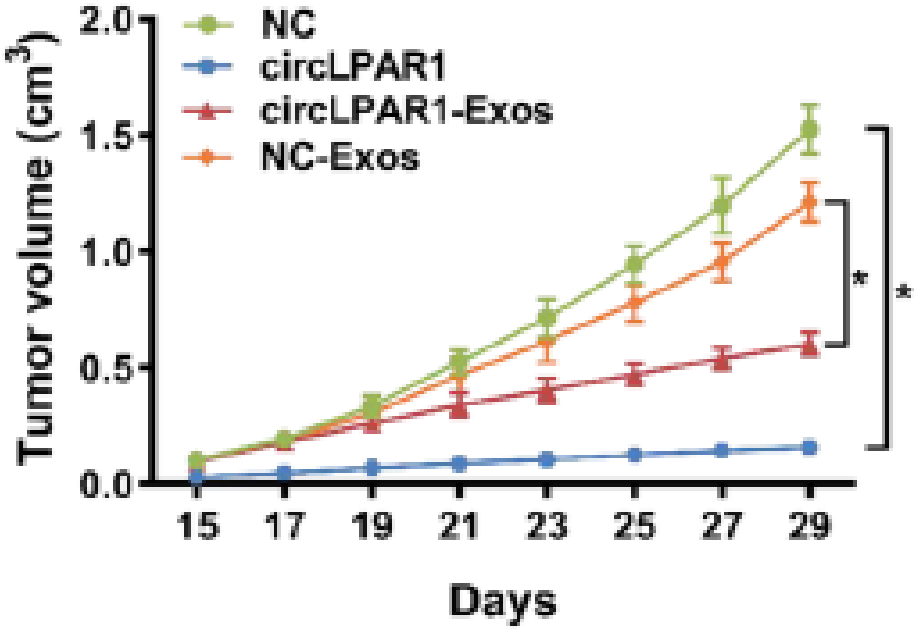
CircLPAR1 suppresses CRC growth *in vivo*

CircLPAR1 overexpression or circLPAR1-Exos treatment affect:

❖ **Tumour weight**



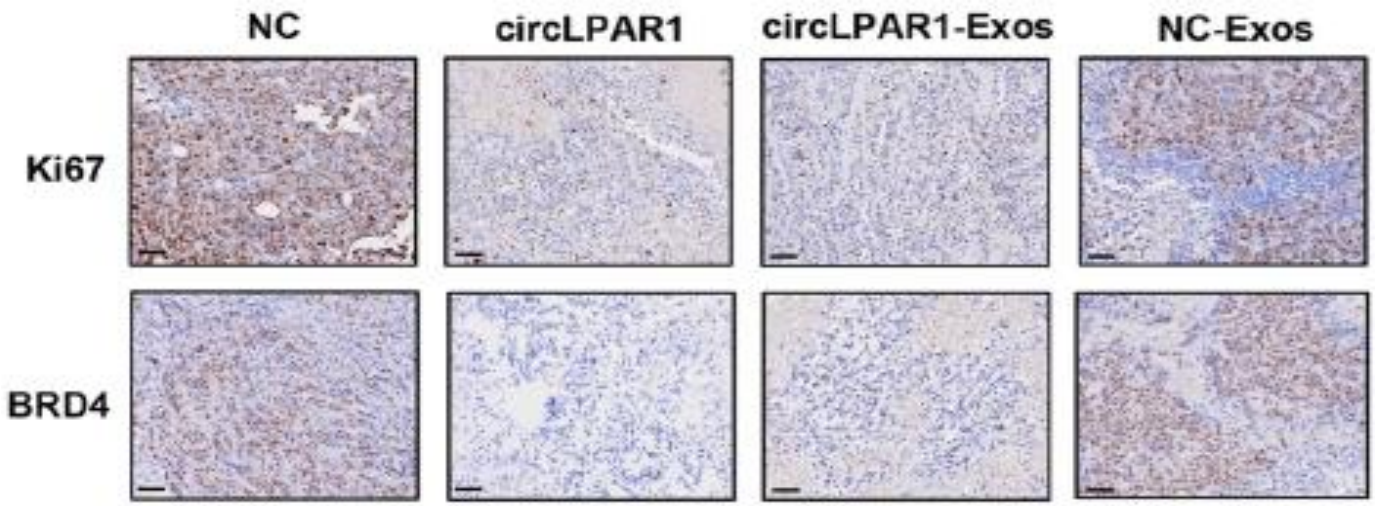
❖ **Tumor size**



CircLPAR1 suppresses CRC growth *in vivo*

CircLPAR1 or circLPAR1 Exos tumour tissues show:

- **Slower growth** compared to control
- *Haematoxylin eosin staining* + *Immunohistochemistry* → **decreased levels of Ki67** (proliferation) and **BRD4**



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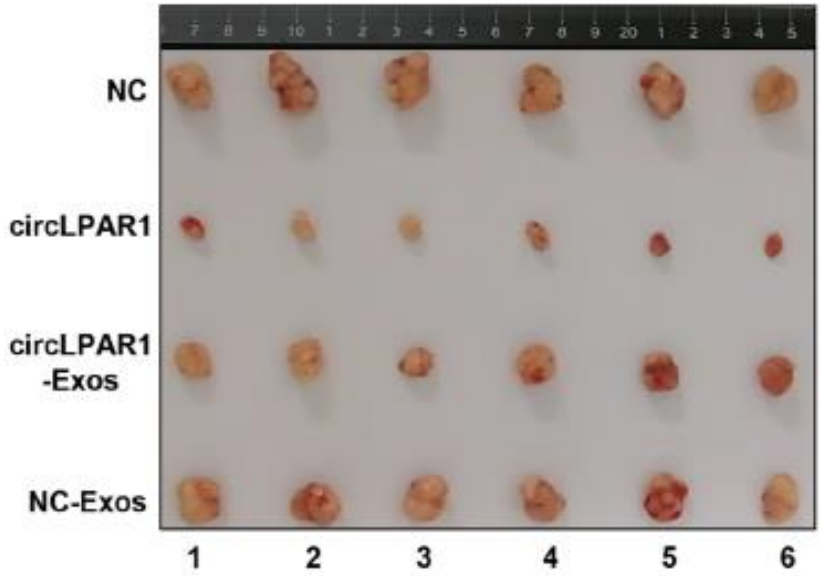


Also *in vivo*, circLPAR1 plays a **tumour suppressing role** in a **BRD4-dependent** way

Outline

Introduction: circRNAs

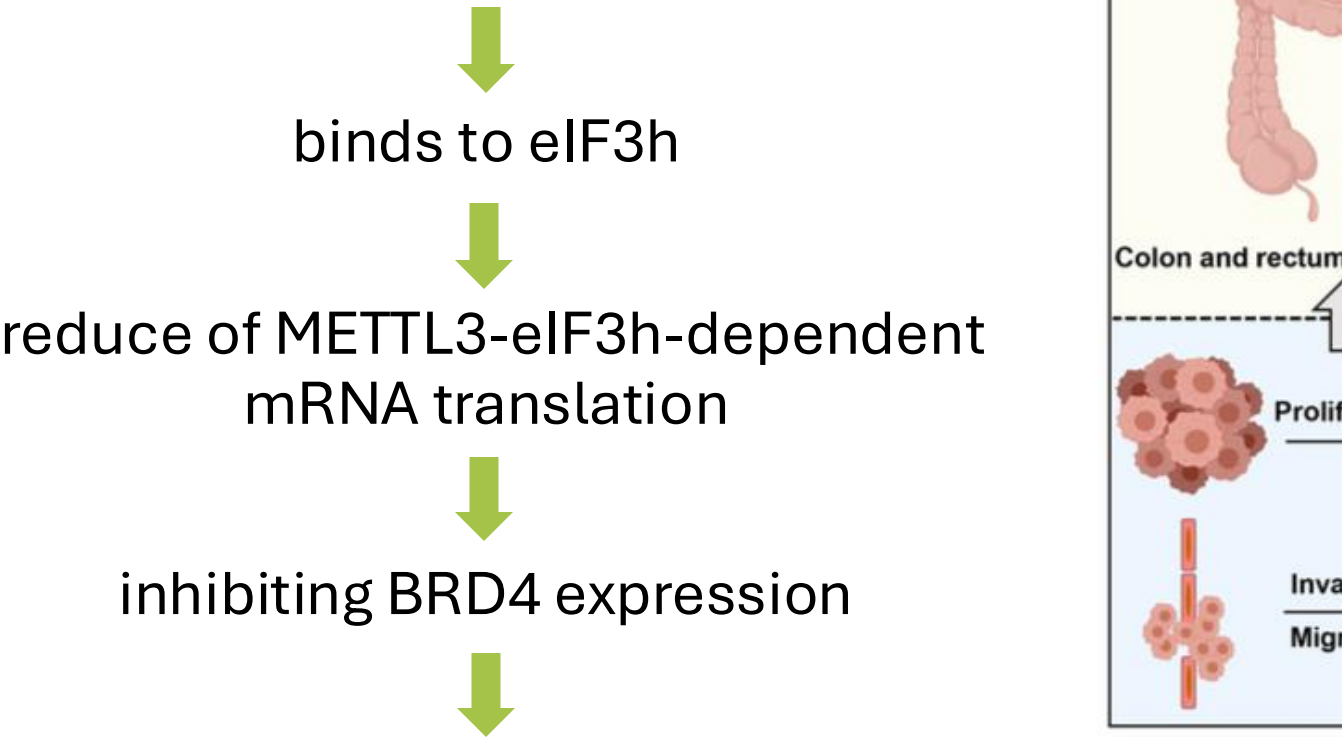
CircLPAR1: experimental results

CircLPAR1: discussion

Conclusions

CircLPAR1: recap

Exosomal circLPAR1: internalized by colorectal cancer cells

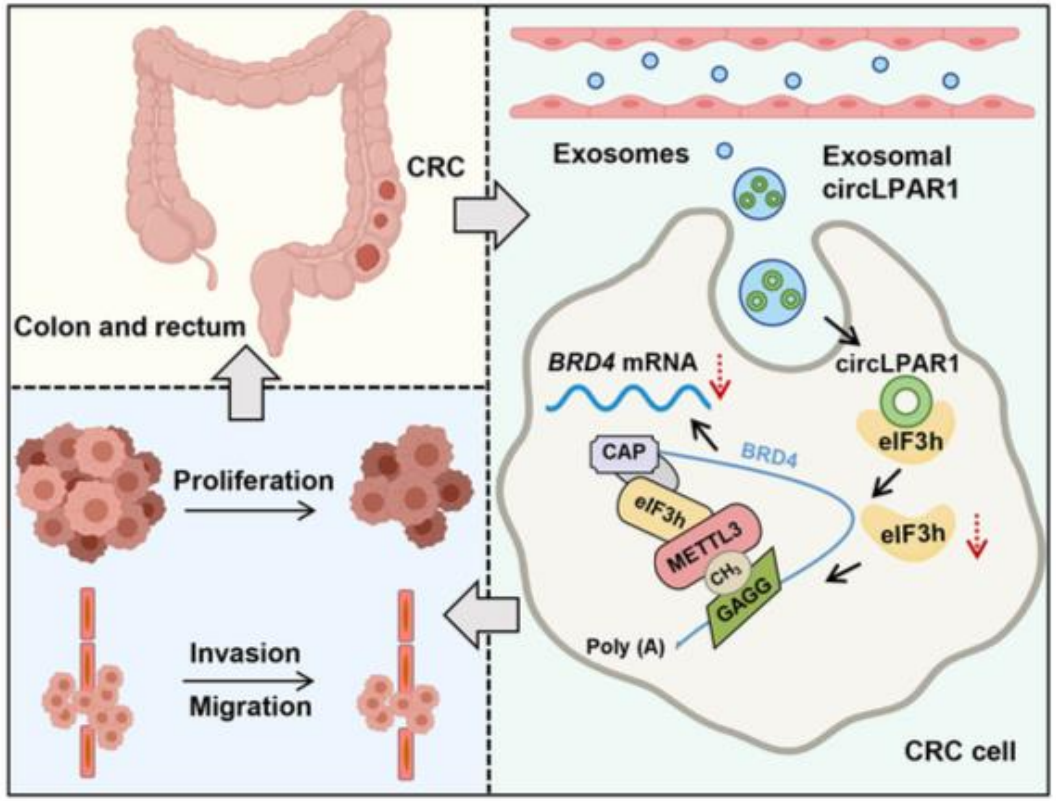


binds to eIF3h

reduce of METTL3-eIF3h-dependent mRNA translation

inhibiting BRD4 expression

suppression of cellular proliferation, invasion and migration



CircLPAR1 as biomarker: the advantages

The advantages of circLPAR1 are based on three aspects:

1. **Stability** in the plasma – encapsulated in exosomes, resistant to actinomycin D and RNase R
2. **Unique for colorectal cancer patients** – the patients can be distinguished from the cancer free ones with acceptable sensitivity and sensibility
3. **Suppression of cancer cellular phenotype** – helpful both for exploring the mechanisms of colorectal cancer and offering new therapeutic approaches

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CircLPAR1: discussion

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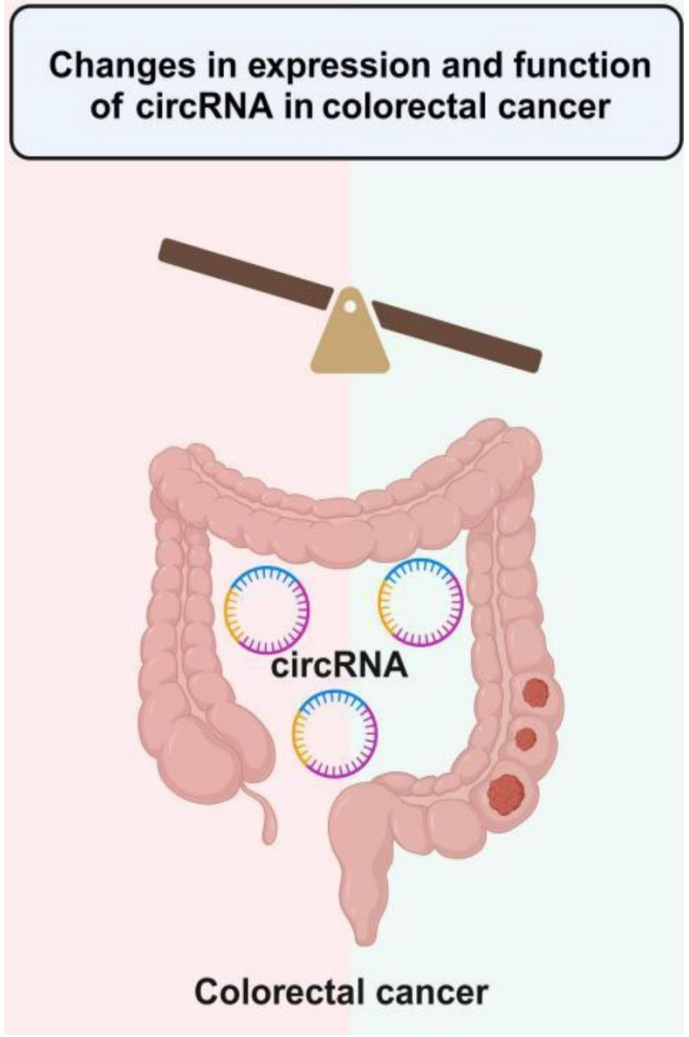
CircLPAR1 is a promising factor in CRC

- BRD4-dependent tumour suppressing role
- Exosomal → potential non invasive liquid biopsy
- Exosomal → taken up by cells
- Stable
- Great specificity and sensitivity
- Known sequence and level variations

<u>Valid biomarker to improve adopted prevention screenings</u>



[Cao et al., 2025]

What's next? Future perspectives

More flexible **screening criteria**: survival and functional analysis

Further validation: **wider cohorts** to be considered

Bioinformatic tools: circRNA prediction to be improved

CircLPAR1 alternatives: Discovery of other circRNAs

CircRNA challenges: Addressing critical issues for clinical applications

CircLPAR1 associated factors: **BRD4 inhibitors** as alternative therapy

Open questions

Direct interaction of circLPAR1 and eIF3h? Or potential "*bridge" proteins*?

Could circLPAR1 also be a **predictive** marker?

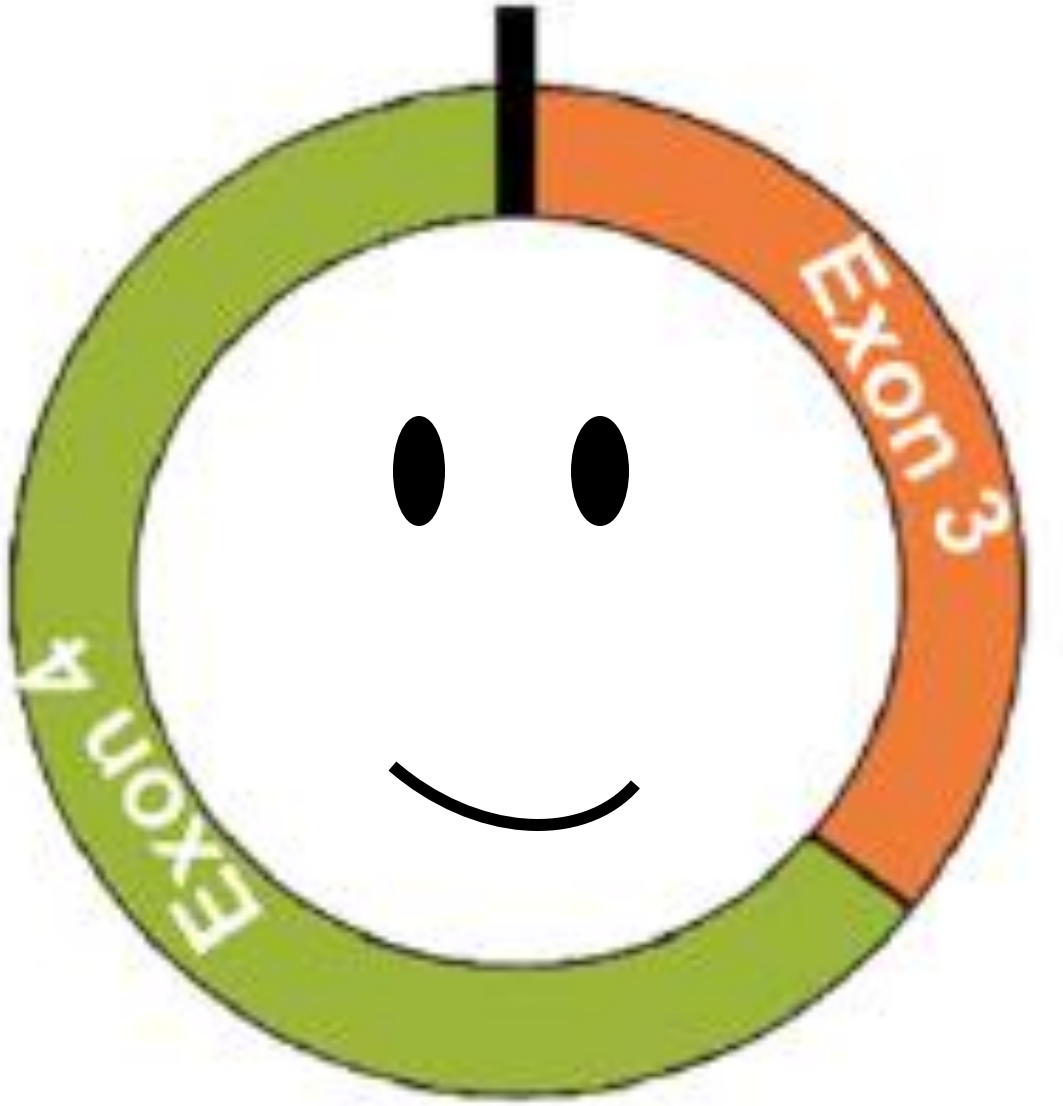
Further validation: Does the oncosoppressing role of circLPAR1 involve **METTL3**?

Is circLPAR1 downregulated also in **sporadic CRC with early onset**?

How does circLPAR1 act on **metastatic ability** *in vivo*?

Why circLPAR1 is downregulated in CRC? Which are the molecular mechanism involved?

Thank you for the attention!



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- **ARTICLE Zheng, R., Zhang, K., Tan, S. *et al.* Exosomal circLPAR1 functions in colorectal cancer diagnosis and tumorigenesis through suppressing *BRD4* via METTL3–eIF3h interaction. *Mol Cancer* 21, 49 (2022)**
- REVIEW Zhang, Y., Luo, J., Yang, W. *et al*. CircRNAs in colorectal cancer: potential biomarkers and therapeutic targets. *Cell Death Dis* 14, 353 (2023)
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Let’s discuss!

Supplementary slides

Back-splicing

→ Proteins and factors that are involved in the regulation of the process

RBPs:

- RBFOXs (1/2/3) – both repress and activate the circRNAs synthesis
- MBL/MBNL1 – activate the circRNAs formation
- QKI, Quaking – activate the circRNAs formation

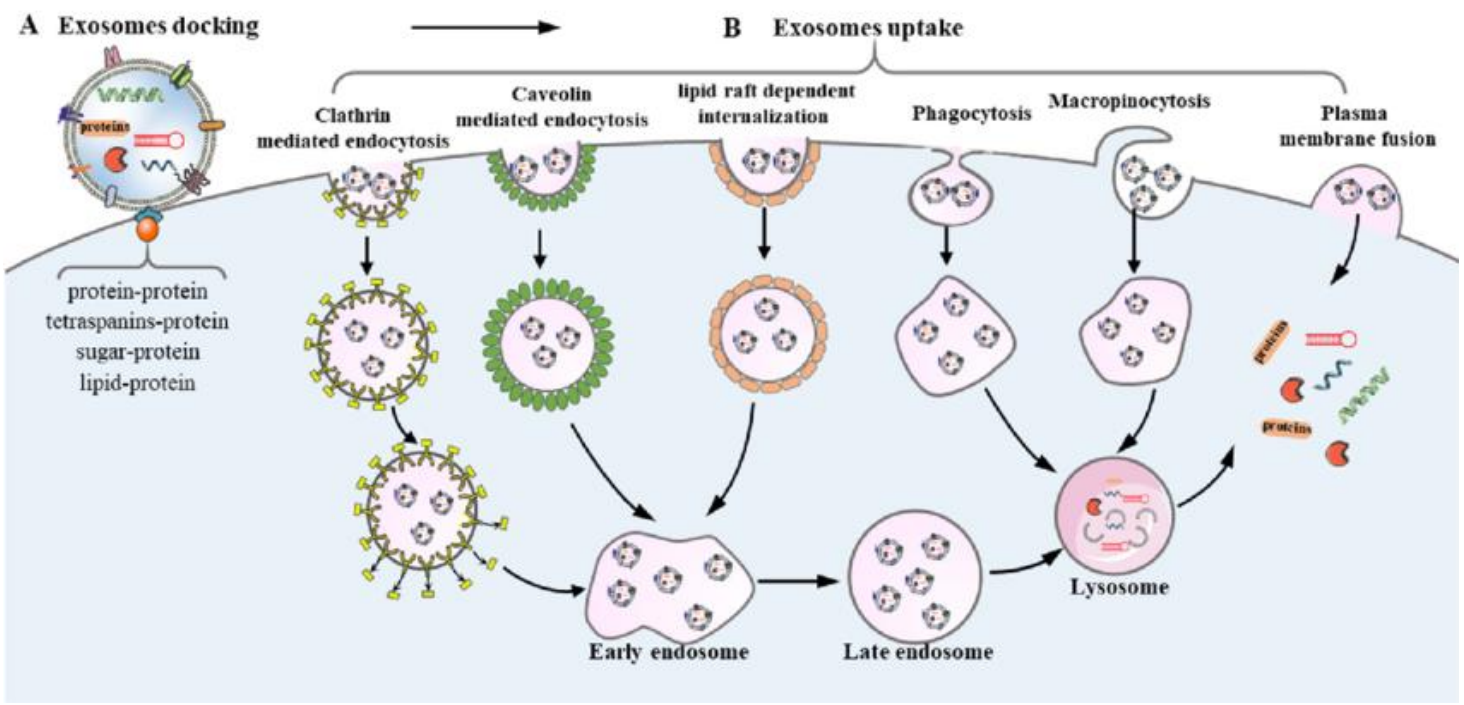
They can dimerize and promote the interaction between 5'ss and 3'ss

Enzymes:

- ADARs – inhibition of circRNAs synthesis
- DHX9 – repressive effects

Uptake of exosomes

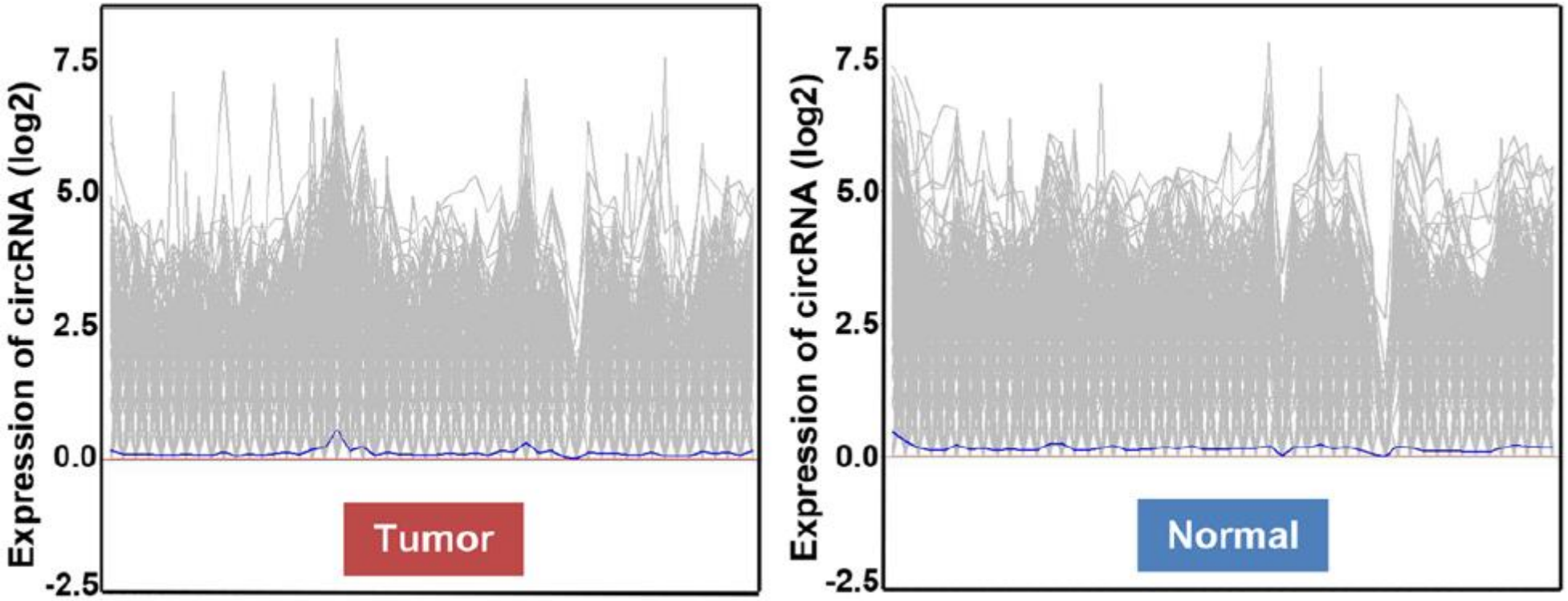
The exosomes may be taken up by recipient cells via the **endocytosis route** (clathrin mediated endocytosis and caveolin-mediated endocytosis), **phagocytosis micropinocytosis**, **receptor-mediated endocytosis**, as well as by **direct fusion** with the plasma membrane, which causes the release of the contents into the cytoplasm



[Zhou et al., 2022]

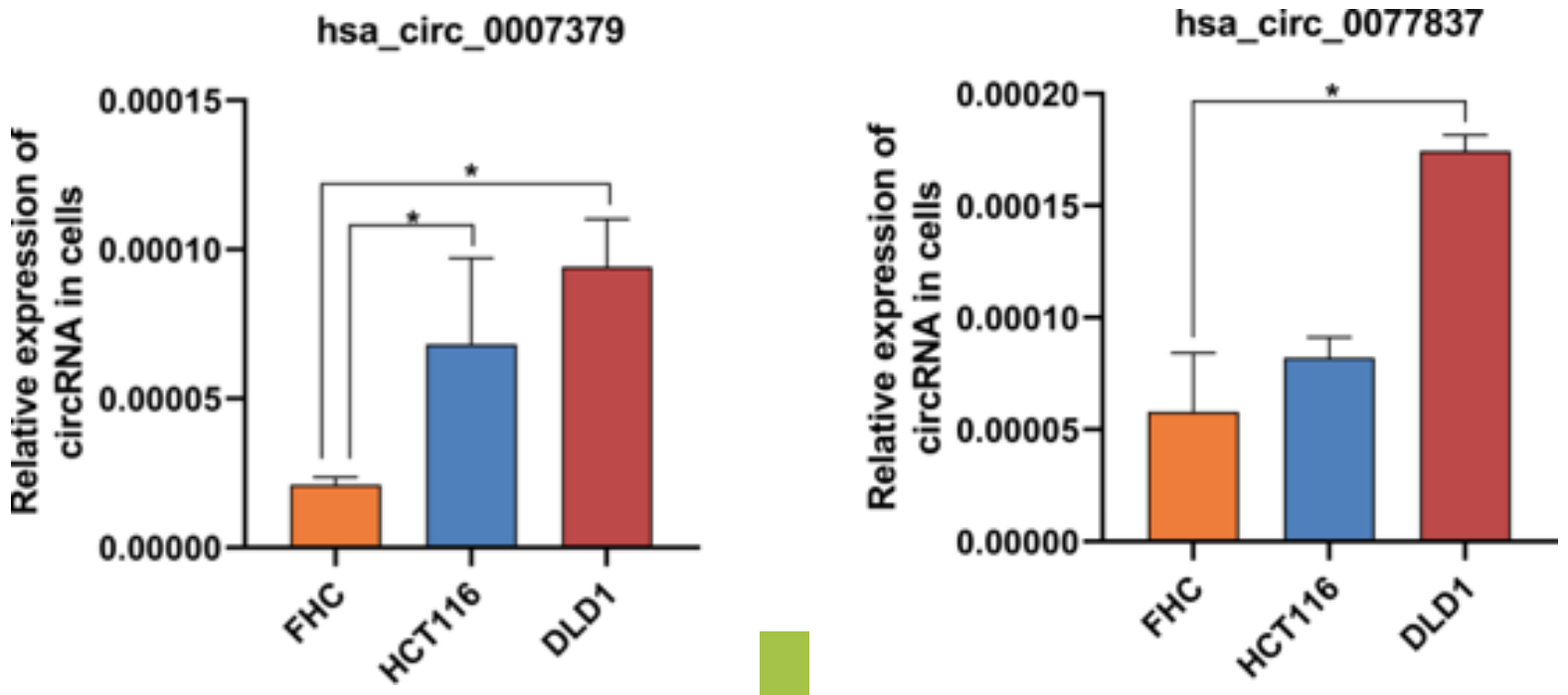
Examples: integrings, mannose receptor, adesion molecules, TIMs receptors

RNA Seq output after circRNAs selection



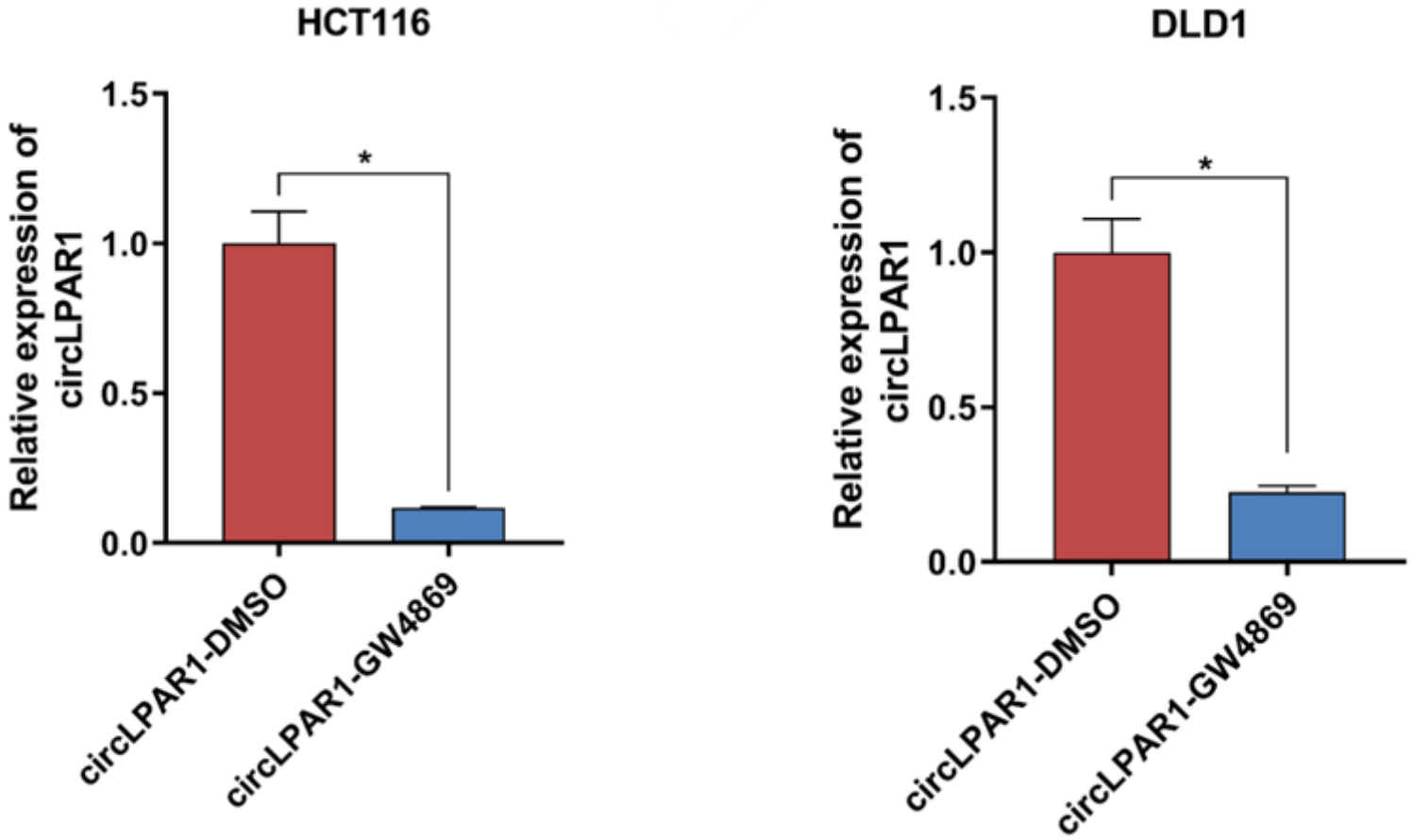
Other exosomal circRNAs levels in cell lines

Selected downregulated circRNAs in tissues *didn't meet the expectations* in cell lines → can't be selected for the downstream studies



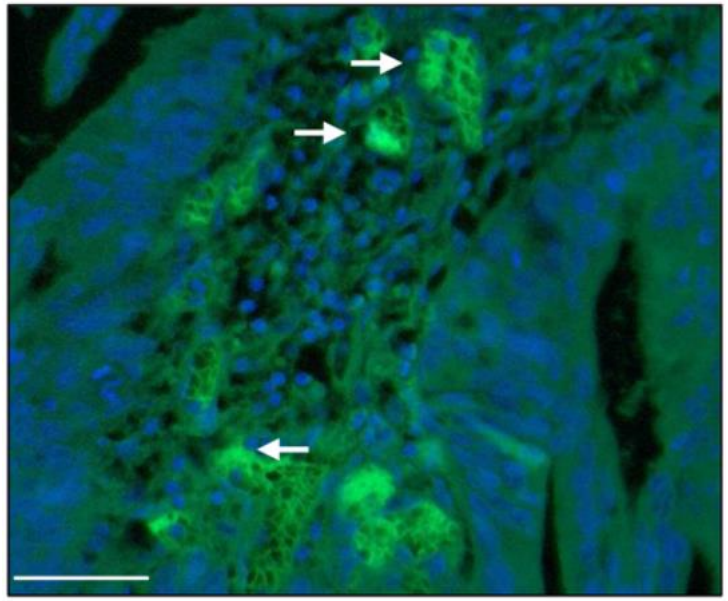
Differences between the tissue (**all cell types** in TME) and the **pure cell line**

Inhibition of exosome secretion leads to decreased level of circLPAR1 in culture medium



Normalized to the negative control (DMSO)

circLPAR1 cellular localization

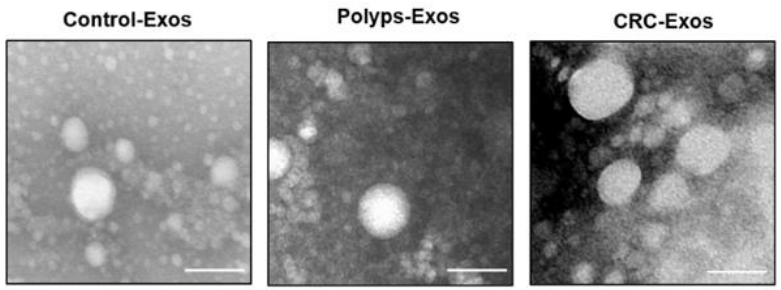


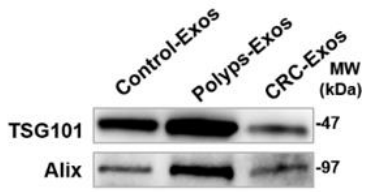
circLPAR1 cellular localization

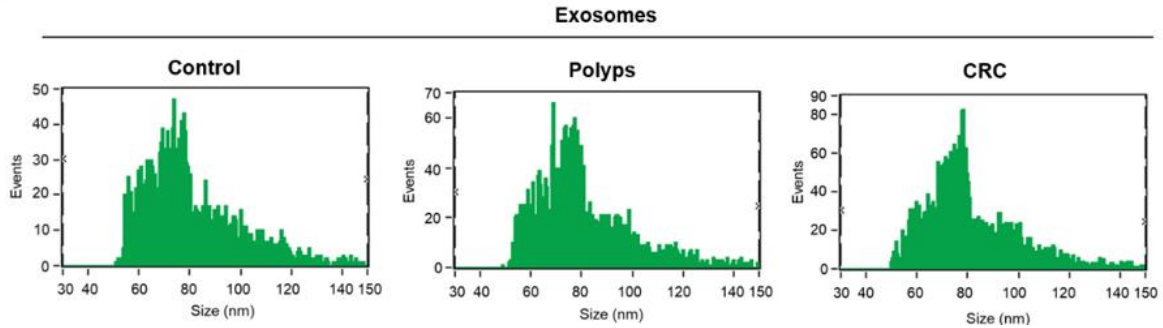
→ Localization of circLPAR1 in colorectal cancer tissue determined by FISH assay

Exosome purification from plasma

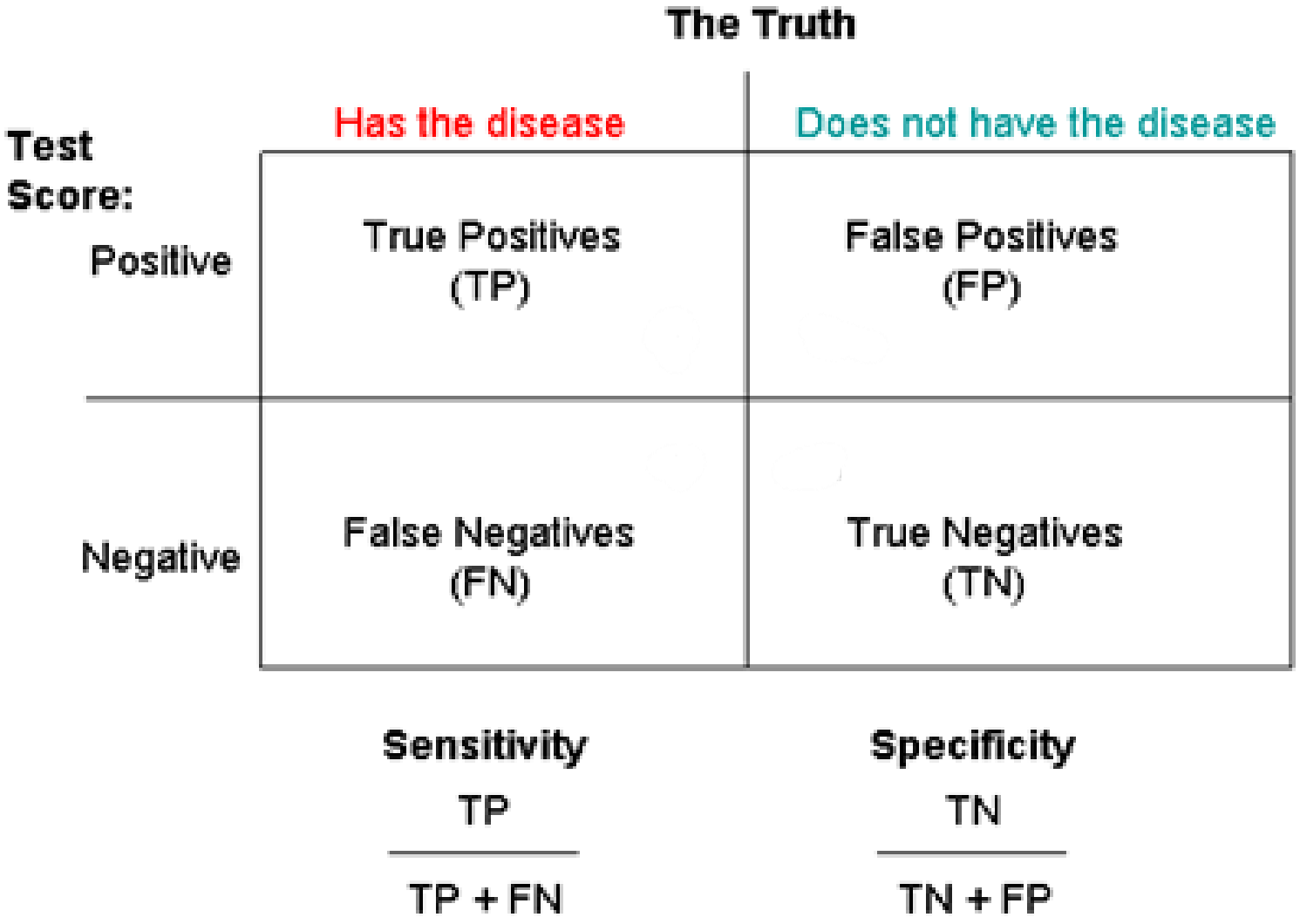
1. Peripheral blood centrifugation 3000 rpm 4° C for 10 minutes → plasma fraction
2. Pretreatment with thrombin and kit for exosome extraction → quantitative exosomes isolation
3. Exosome characterization







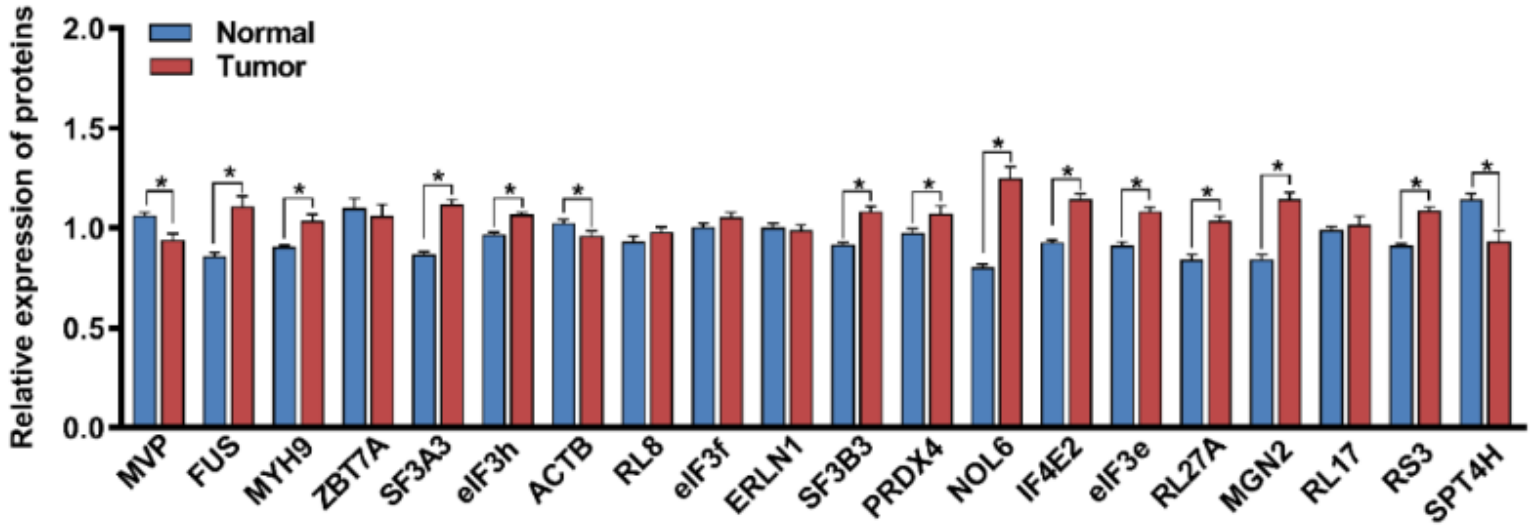
Sensitivity and specificity



Optimal to increase:

- **Sensitivity** if the *disease is curable* → *avoid false negatives* to start immediately the therapy on all patients
- **Specificity** if available therapies *don't cure the disease* → *avoid false positives* to NOT start harmful treatment on healthy individuals

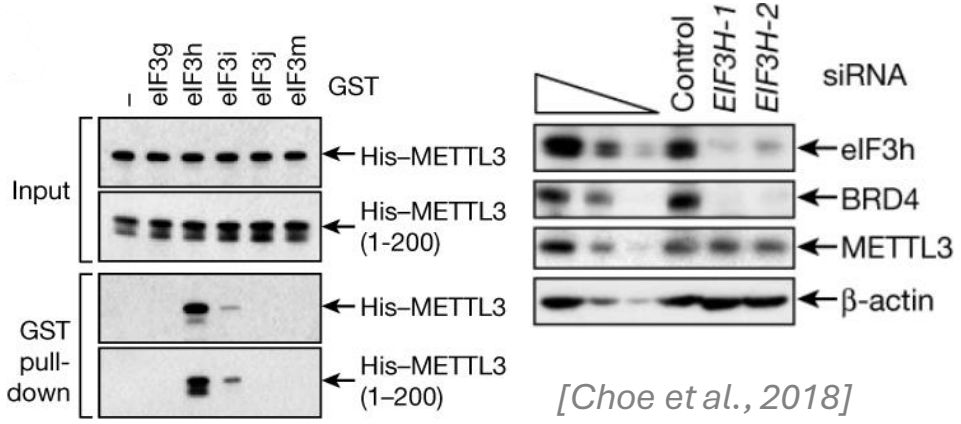
circLPAR1 and RBPs interaction



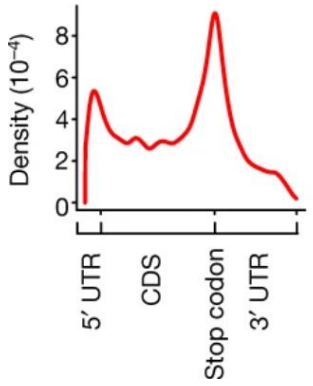
The 23 RBPs candidates identified by pull-down assay for the interaction with circLPAR1

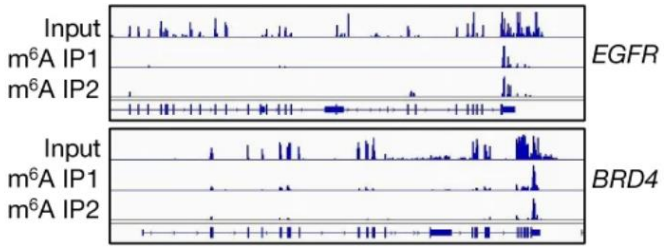
eIF3h-METTL3 direct interaction

1. Reporter mRNA assay with different MS2 positions → METTL3 (1-200 aa) **binds to 3'UTR**, close to the translation stop codon
2. Far Western Blot with recombinant METTL3 and EIF subunits+ GST pulldown+PLA → **direct interaction METTL3-eIF3*h***
3. eIF3h KO → no enhanced translation by METTL3



[Choe et al., 2018]





[Choe et al., 2018]

4. KO of METTL3 or eIF3h → decreased BRD4 protein levels
5. meRIP-Seq → *GGAC motif* enriched in **m^{6}A**
6. Metagene analysis: close to the stop codon
7. Gene Ontology analysis → BRD4 and EGFR are enriched in m^{6}A

CircRNA challenges

CircRNAs in therapy

- *circRNA levels < miRNA levels* → hard to reach the **stoichiometric requirement** for sponge effects
- Overexpression to be avoided
- Production of **highly purified** circRNAs
- Limited studies on circRNAs

No circRNA in clinical trials

CircRNAs as markers

- *Limit of* ***detection***: high sequence similarity linear mRNA → choice of qRT-PCR primer is critical
- *Microarray limitation*: circRNAs need **to be known** in advance
- CircRNAs differently expressed in tissues *but* not present in blood/serum
- Lack of sensitivity and sensibility
- Need for **standardized methods**

Some hypothesis of circLPAR1 downregulation in CRC

- Downregulation or mutation of specific **receptors** for endocytosis of exosomes
- Overproduction and release of exosomes
- Mutation or downregulation of **splicing factors** involved in back-splicing
- Upregulation of splicing factors that promotes linear splicing
- Upregulation of specific endonucleases for the **degradation of circLPAR1**
- Hypermethylation of the **promoter** of LPAR1 gene