Liquid biopsy, as the source of cell-free nucleic acid for the diagnosis, treatment, and monitoring of cancers

Beáta Soltész, Orsolya Biró, János Lukács, Gréta Gombos, Nikolett Németh, Zoárd Krasznai, Róbert Póka, András Penyige, István Balogh, Bálint Nagy

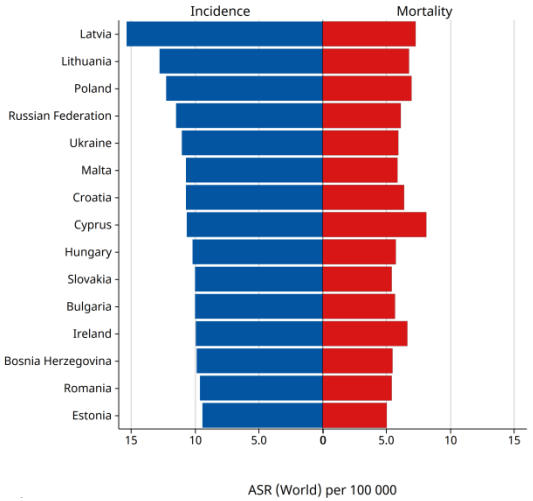
6th Central - Eastern European congress
on cell free DNA and medical practice

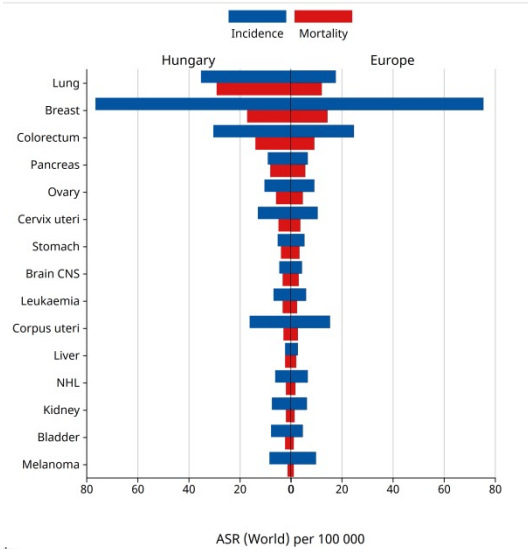
7-8 March 2024

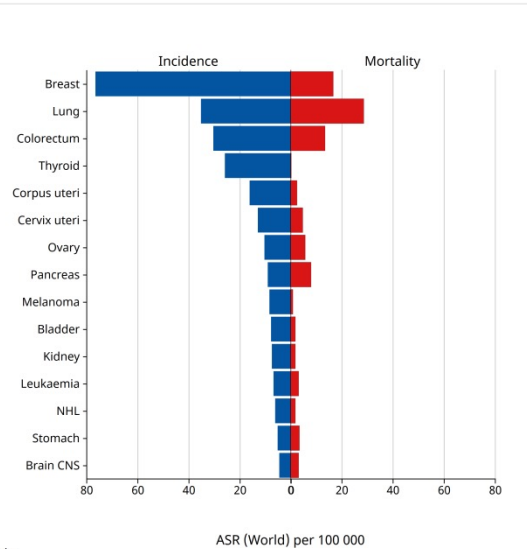
DEBRECENI EGYETEM

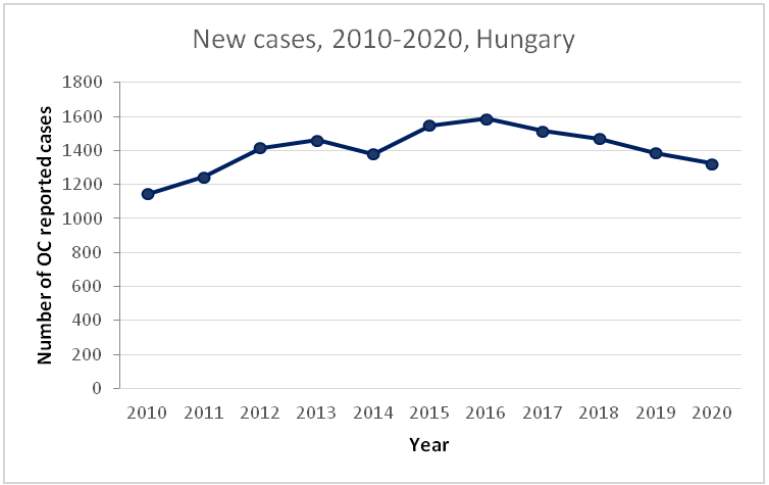
Why?

Age-standardized Rate per 100 000, Incidence and Mortality, Females, in 2022









Hungarian tumor register

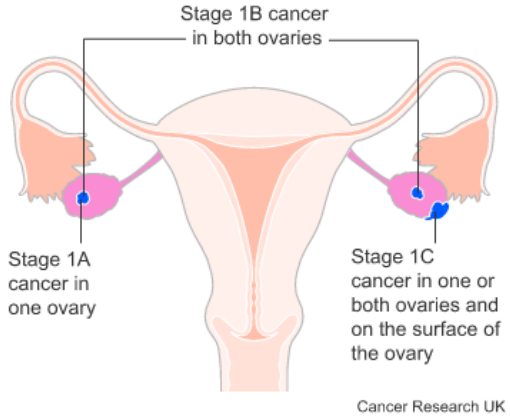
- The fifth most common cause of cancer death among women
- 1200-1300 new cases / year (739 death in Hungary in 2014)
- 5-year survival rate: 30-90%

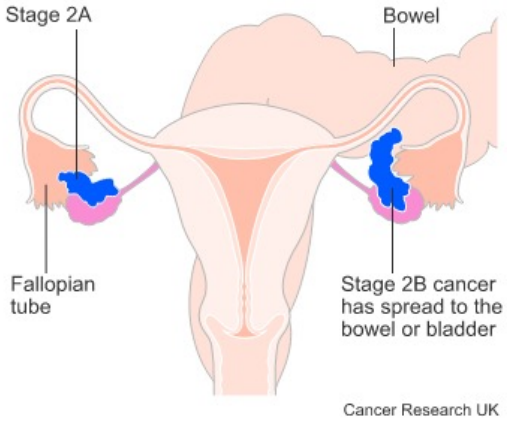
DEBRECENI EGYETEM

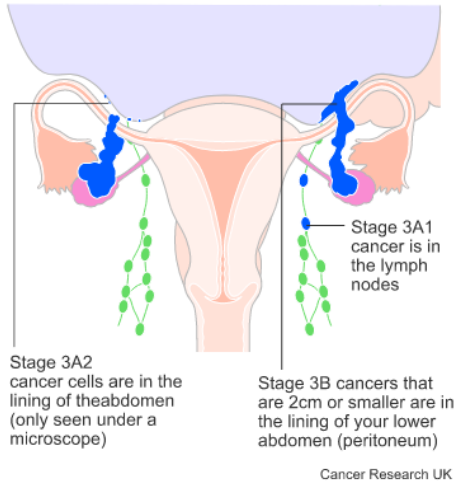
Ovarian cancer

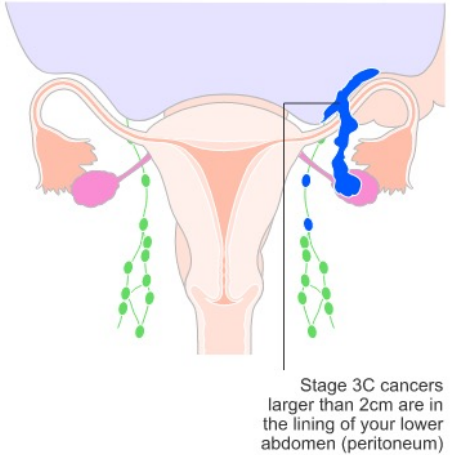
- About 4% of all tumours
- 4.2% of all deaths
- Diagnosis:
- -Physical examination
- -Transvaginal ultrasound
- -Hystopathology examination (gold standard)
- -Biomarkers: CA125, HE4
- FIGO staging
- Late diagnosis: no real specific syndromes, no effective screening and early diagnostic methods to detect it
- Difficult to treat
- Biomarker research (early detection and monitoring of treatments)

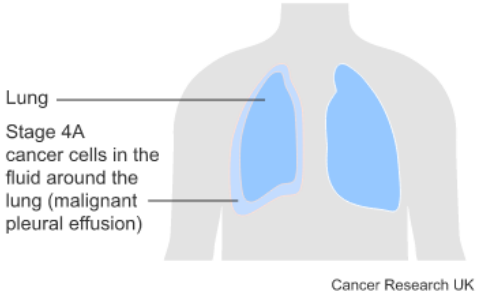
DEBRECENI EGYETEM

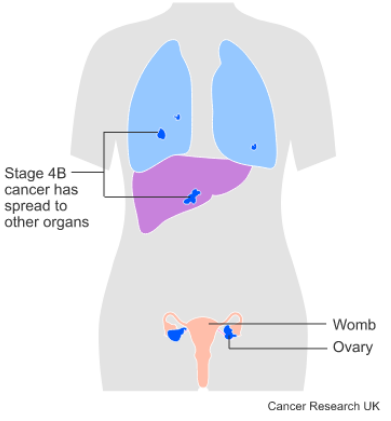












Liquid biopsy

Non-Invasive or minimally invasive

Quick

Easily obtained

Minimal pain/risk

Not so expensive

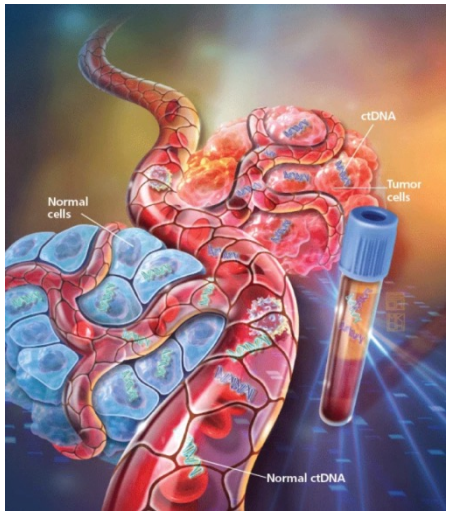
Comprehensive tissue profile

Before and after tumor localization

Applicable in the case of small tumors

In early diagnosis

Repeatable



Tissue biopsy

Invasive

Time-intensive procedure

Not Easily obtained

Some pain/risk

Expensive

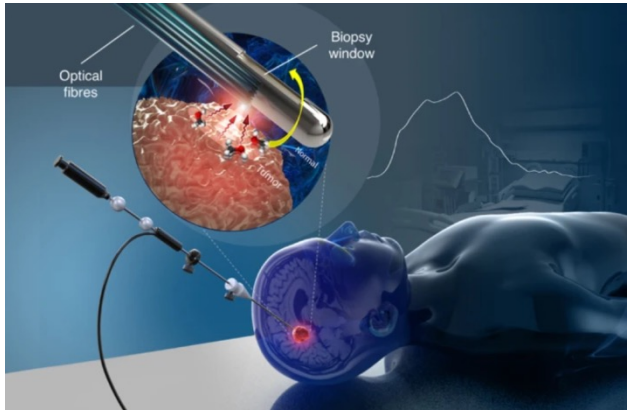
Localized sampling of tissue

Only after tumor localization

Not applicable in the case of small tumors

Limited application in early diagnosis

Not-repeatable

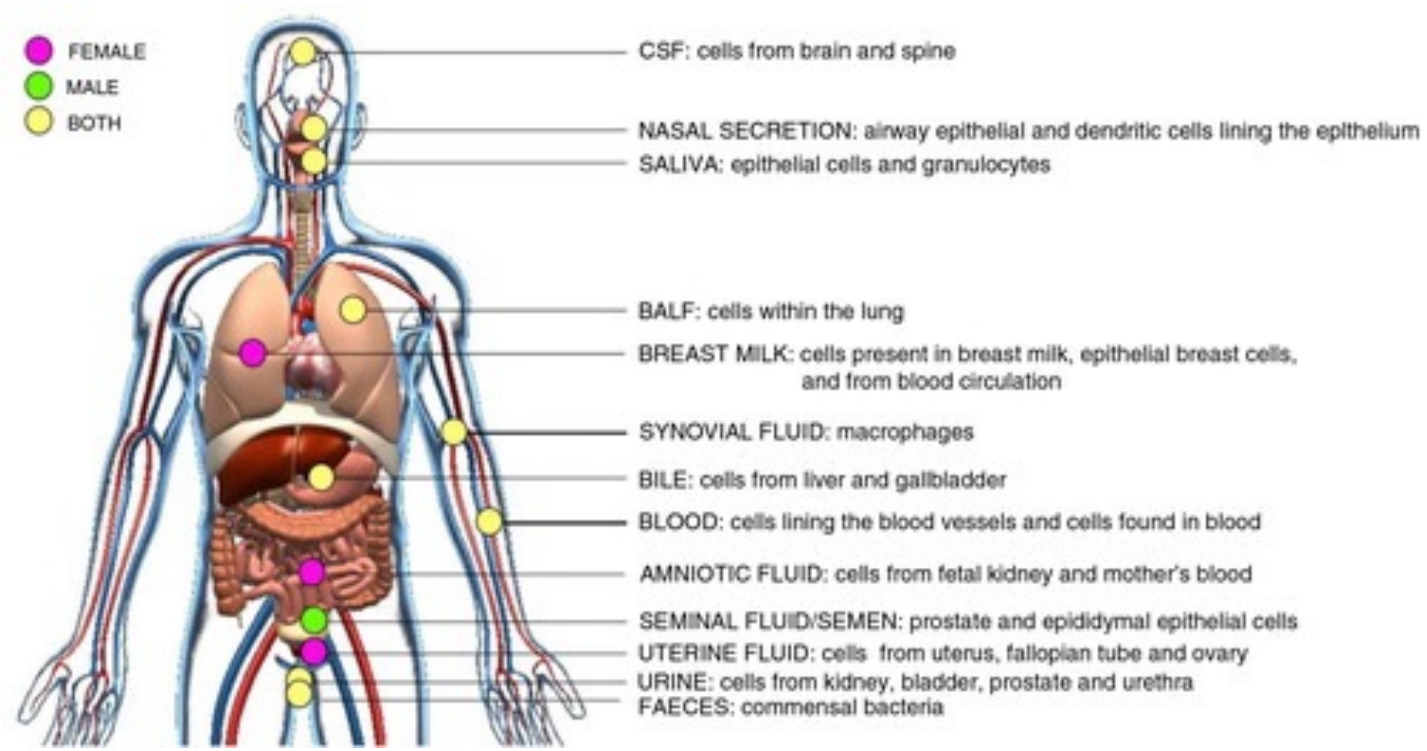


DEBRECENI EGYETEM

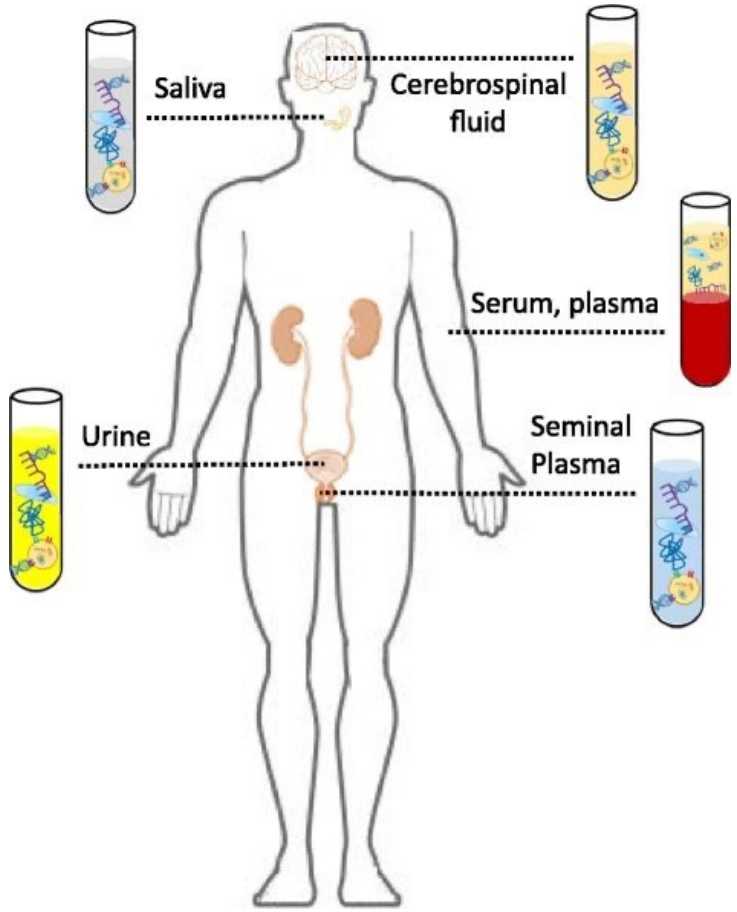
Komatsubara, Sacher. Oncology. 2017;31(8):618-27.

doi: 10.1038/s41598-018-20233-3

Biological fluid



Yáñez-Mó et al. J Extracell Vesicles. 2015;4:27066

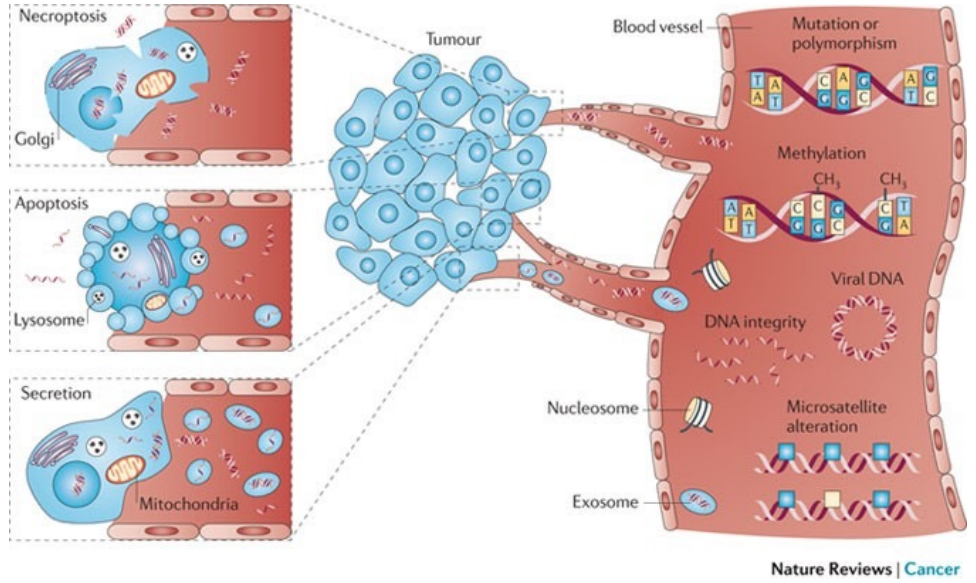


Heredia-Soto et al. Cancers (Basel). 2021;13(8):1986.

DEBRECENI EGYETEM

Cell-free nucleic acids (cf-Nas) and origins

- Cf-nuclear DNA (genomic DNA fragments)
- Cf-mtDNA
- Cf-ctDNA
- Cf-microbial DNA
- Cf-coding RNA (mRNA)
- Cf-non-coding RNA (miRNA, lncRNA, cirrRNA)
- Other RNAs (piwi-RNA, YRNA, Vault RNA)



Schwarzenbach et al., Nat Rev Cancer. 2011;11(6):426-37.

Passive
- cellular lysis
- apoptosis, necroptosis

Active
- exosomes (40-100 nm)
- microvesicles (50-3000 nm)
- associated with lipoproteins (HDL, LDL)
- associated with ribonucleoproteins (e.g. argonaute 2)

DEBRECENI EGYETEM

Materials and methods

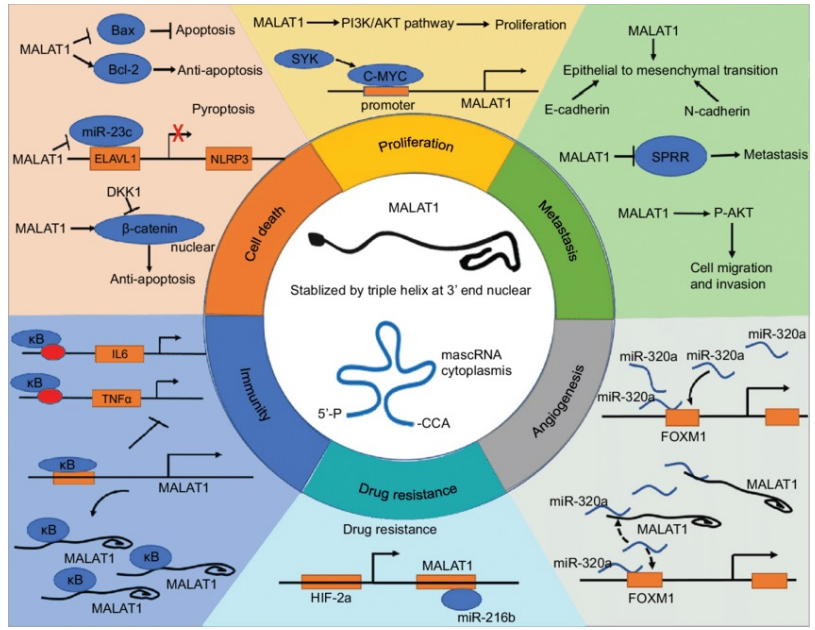
- Ovarian cancer patients (8) and non-cancerous controls (8)
- RNAs and non-coding RNAs were isolated from plasma, plasma-derived exosomes (miRNeasy Serum/Plasma Kit, RNeasy Plus Kits) and tissue (NucleoSpin RNA kit)
- cDNA was synthesised and the level of expression was determined by qRT-PCR (miRCURY LNA miRNA system for miRNAs, RT^2 Profiler PCR system for lncRNAs, DNA Master Hybprobe kit for CD24)
- Exosomes were extracted from plasma (miRCURY™ Exosome Isolation kit), and quantified by Exo-TEST ELISA kit.

DEBRECENI EGYETEM

LncRNA

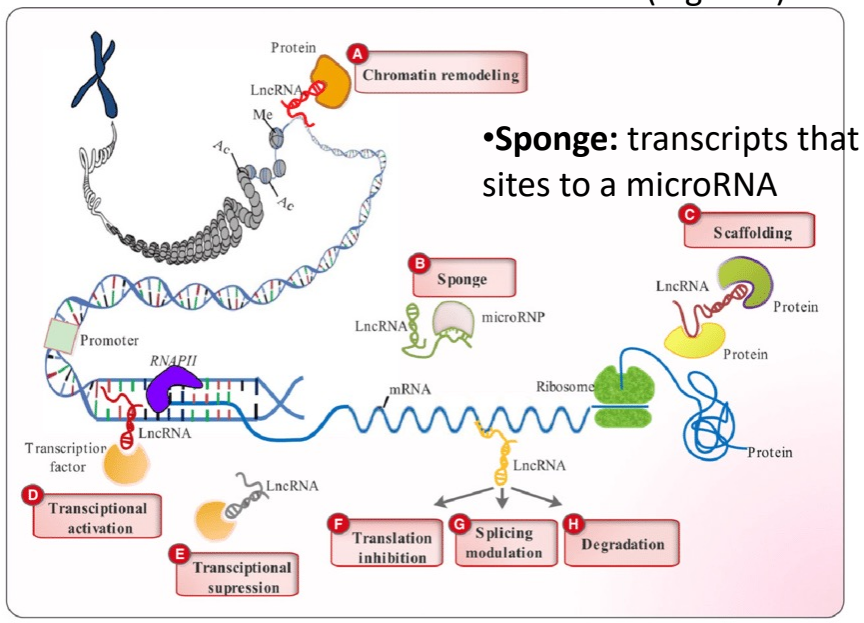
- 200 bp long non-coding RNA molecules
- Origin:
  - Intergenic regions
  - Sense, antisense
  - Intronic
  - Mature RNAs

MALAT1 (metastasis associated lung adenocarcinoma transcript 1): aberrant expression in metastasis



Li et al. Cancer Manag Res. 2018;10:6757-6768.

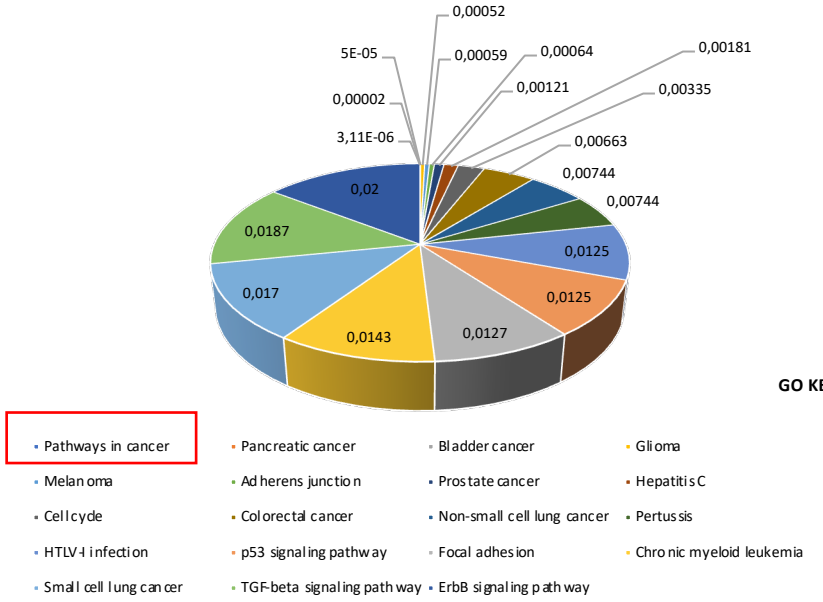
•**Epigenetic modifications:** chromatin modification (e.g. Xist)



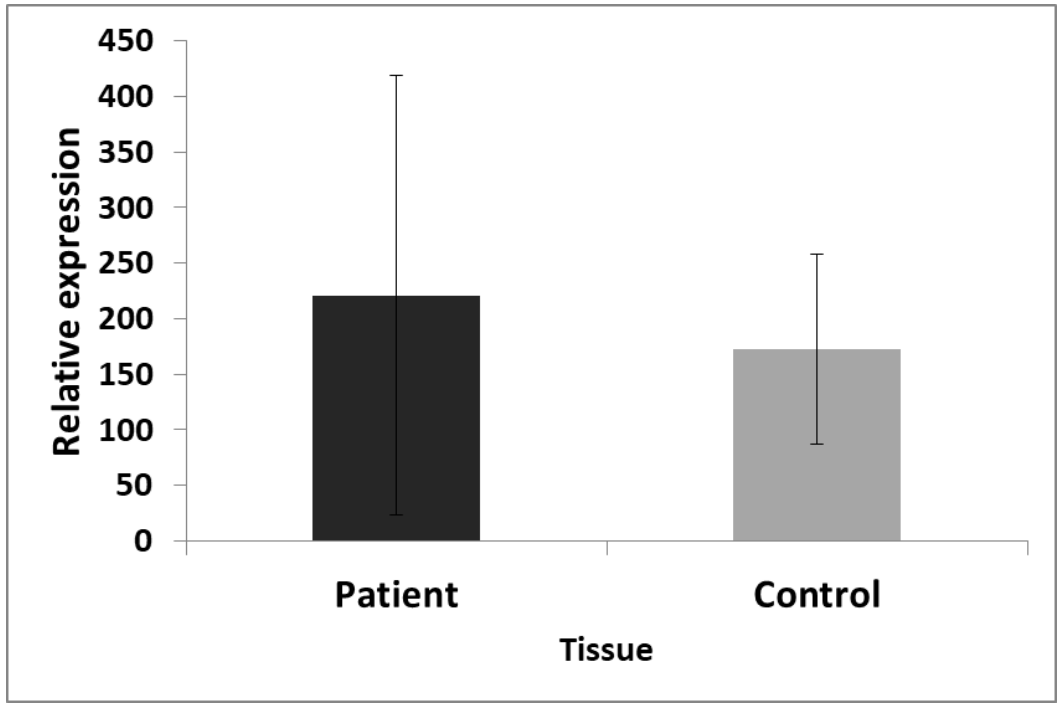
•**Sponge:** transcripts that have tandem binding sites to a microRNA

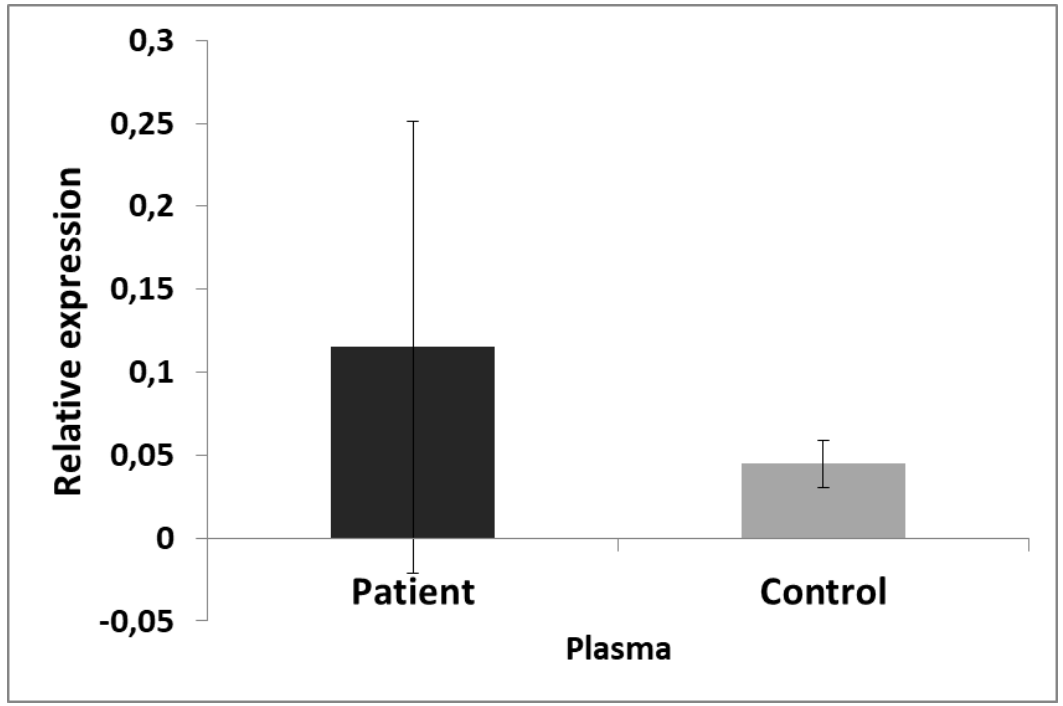
•**Scaffold:** keeping proteins together

Salehi et al. J Cell Mol Med. 2017;21(12):3120-3140



MALAT1 gene expression

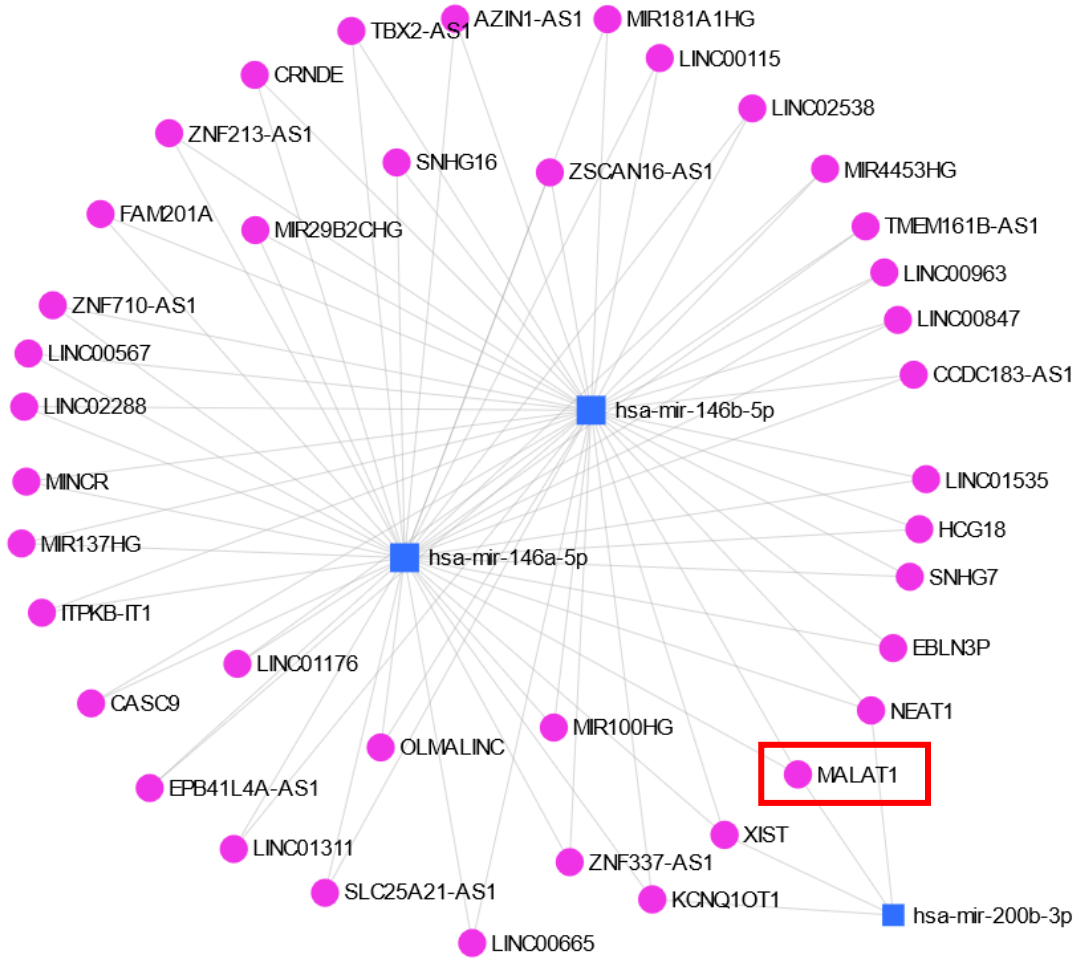




DEBRECENI EGYETEM

Soltész B et al., unpublished

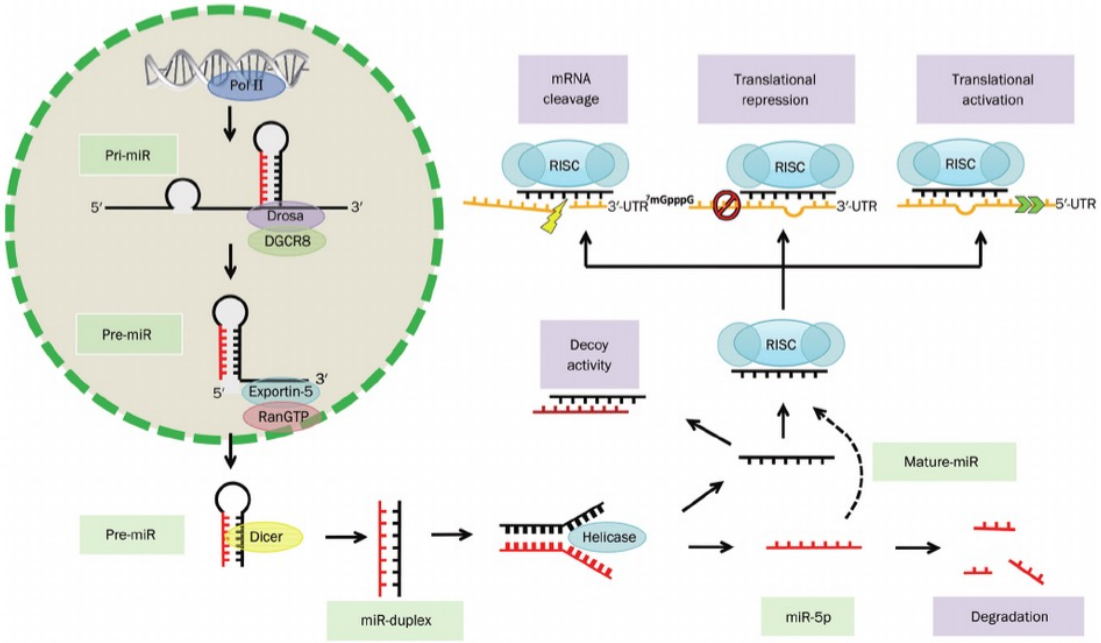
Interaction network of microRNAs and MALAT1

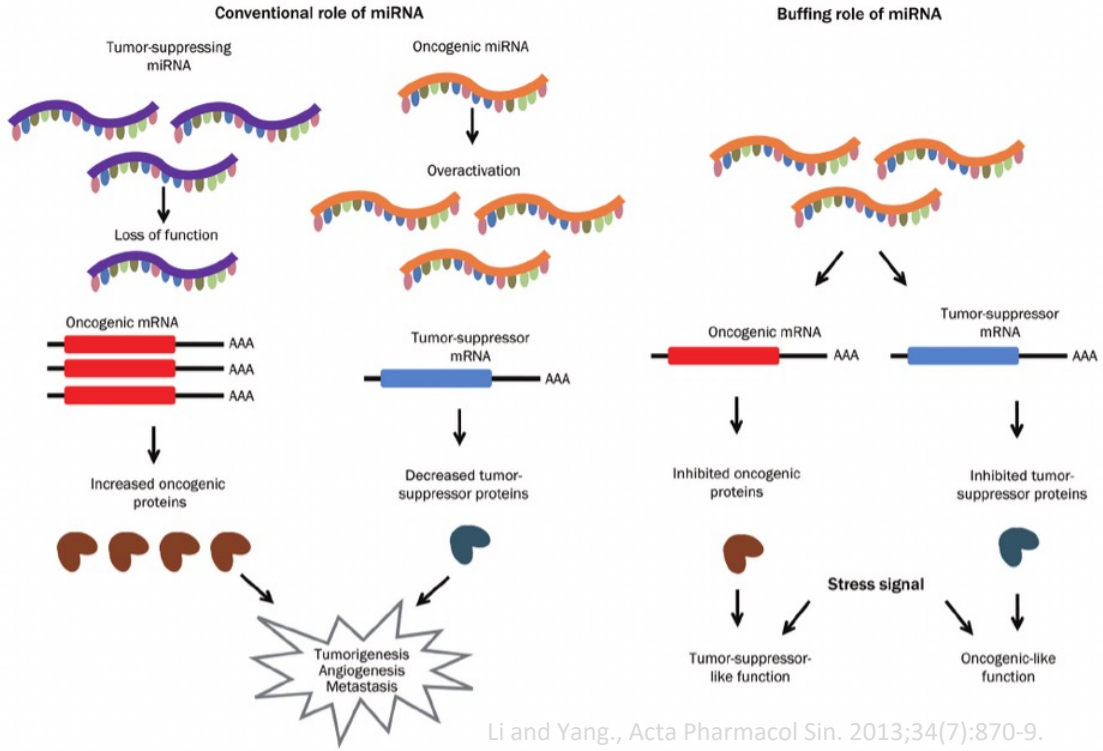


miRNet

MicroRNA

- 18-22 nucleotide long, non-coding RNA molecules
- Present in blood, saliva and other biological fluids
- Gene regulation, mRNA cleveage, translational repression and activation
- Aberrant miRNA expression leads to many diseases (e.g. cancer)





Cell-to-cell communication

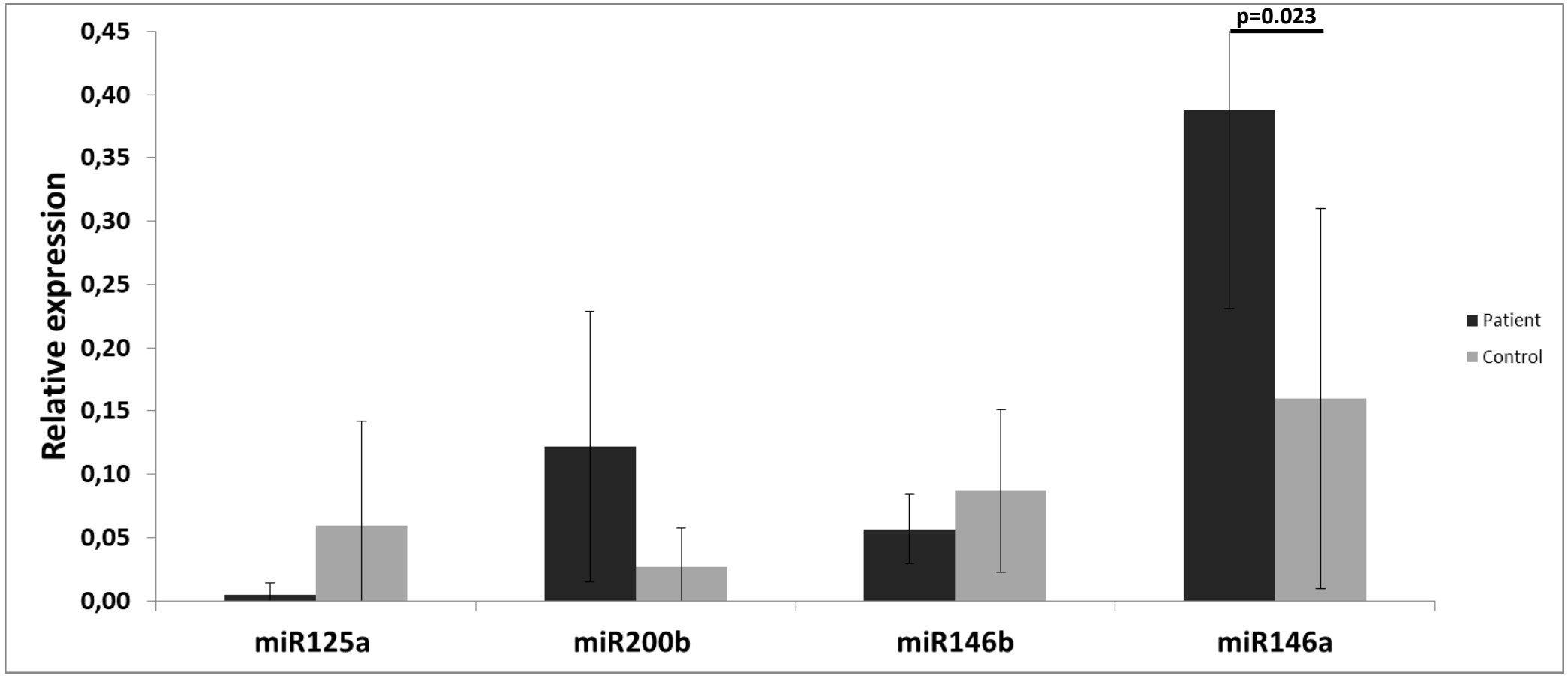
- Non-coding RNAs can regulate gene expression in other cells

It affects the tumour microenvironment

- Stimulate tumour growth through ECM formation and growth factor production
- Induces angiogenesis
- Inhibits immune cells

Li and Yang., Acta Pharmacol Sin. 2013;34(7):870-9.

microRNAs in plasma

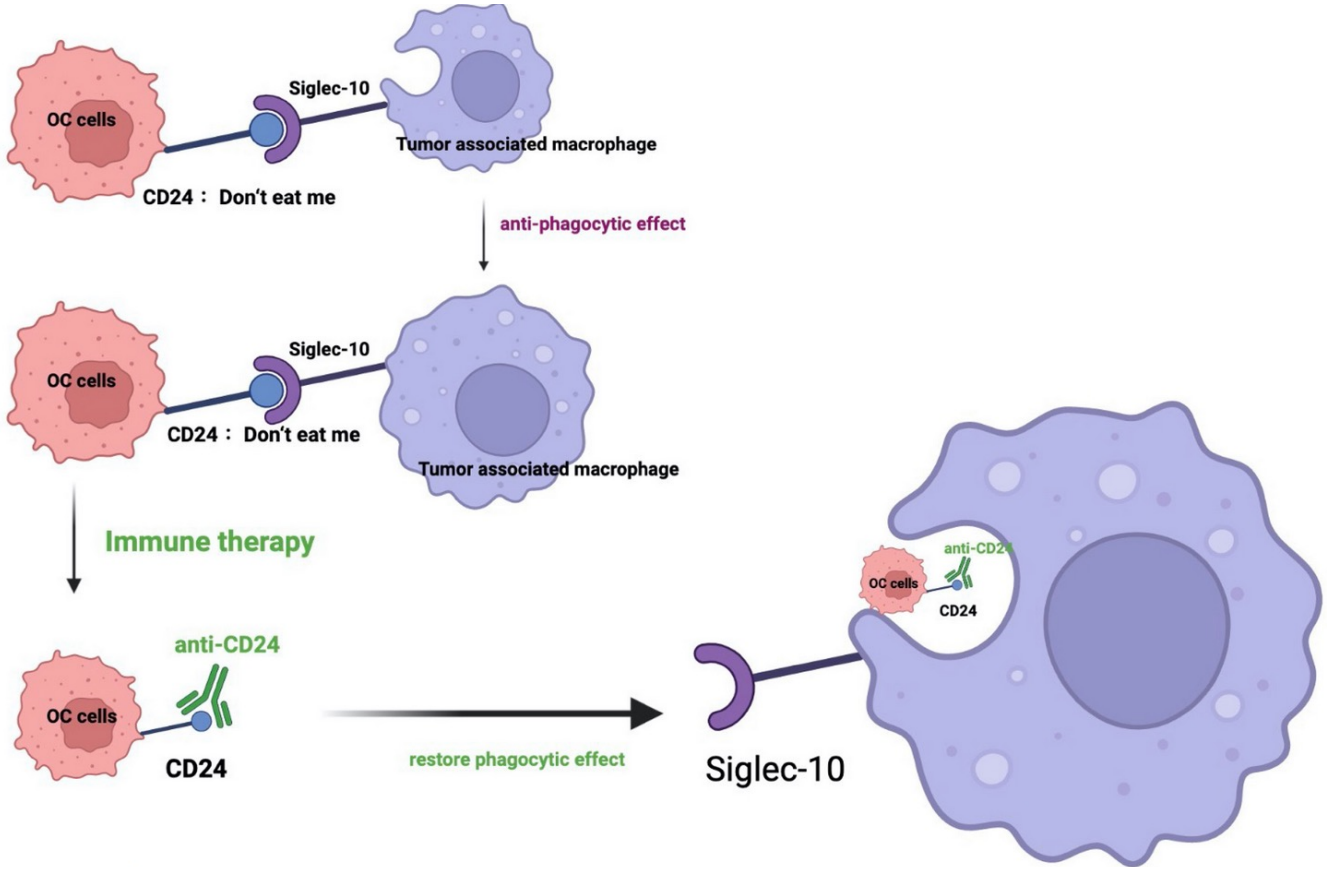


DEBRECENI EGYETEM

Soltész B et al., unpublished

CD24

- a cell surface protein similar to mucin, highly glycosylated
- CD24+ tumour cells in the development of metastases
- CD24 may be an independent prognostic marker in ovarian, prostate and non-small cell lung tumours (Choi et al., 2005)

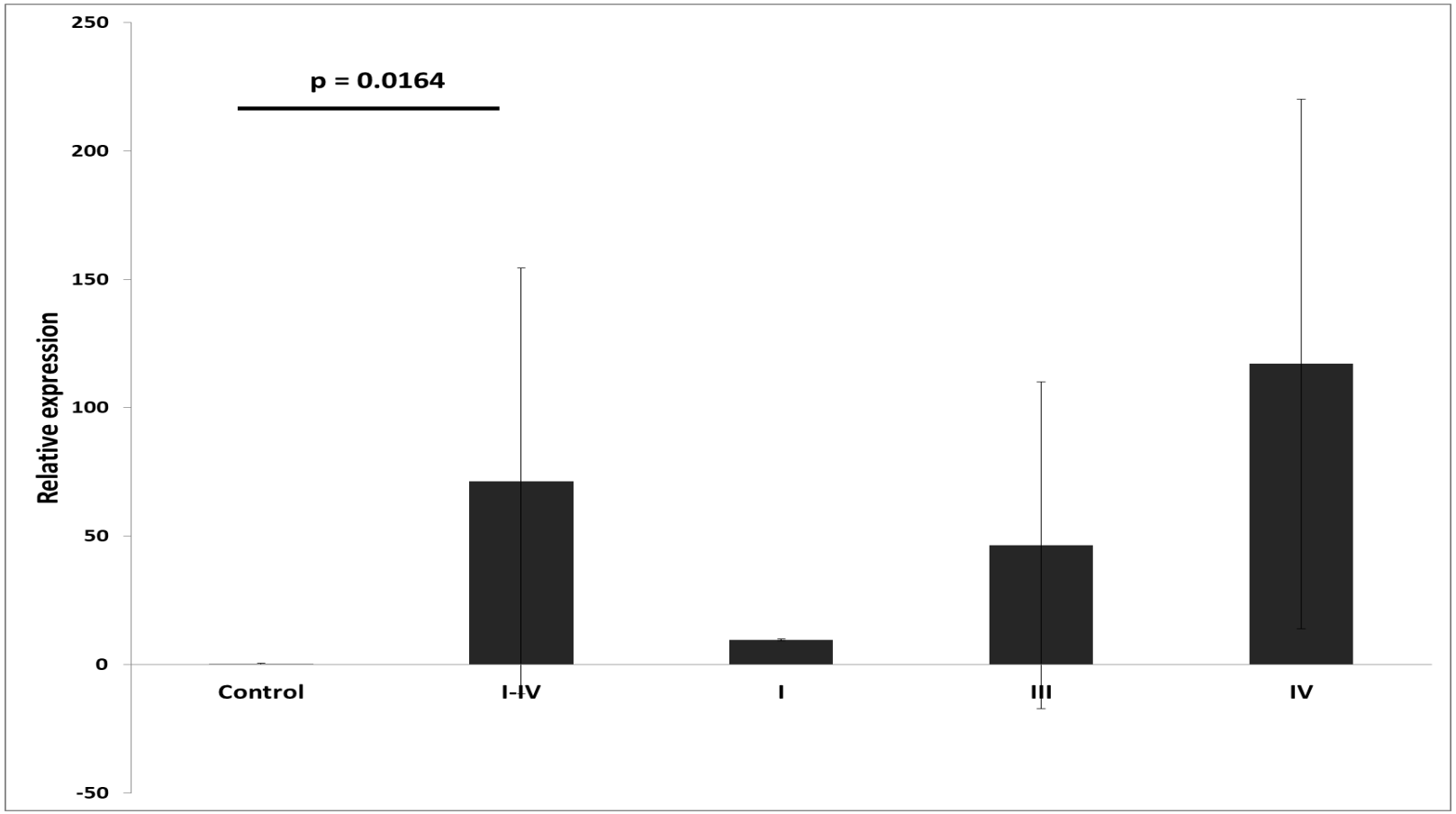


Gu et al., Front Immunol. 2023;14:1183285.

DEBRECENI EGYETEM

CD24 is identified as a novel functional target of miR-146a and ectopic expression of CD24 abrogates miR-146a driven potential Cancer Stem Cell phenotype (Ghuwalewala et al., Front Oncol, 2021:11:651692.)

CD24 expression (in OC tissue)

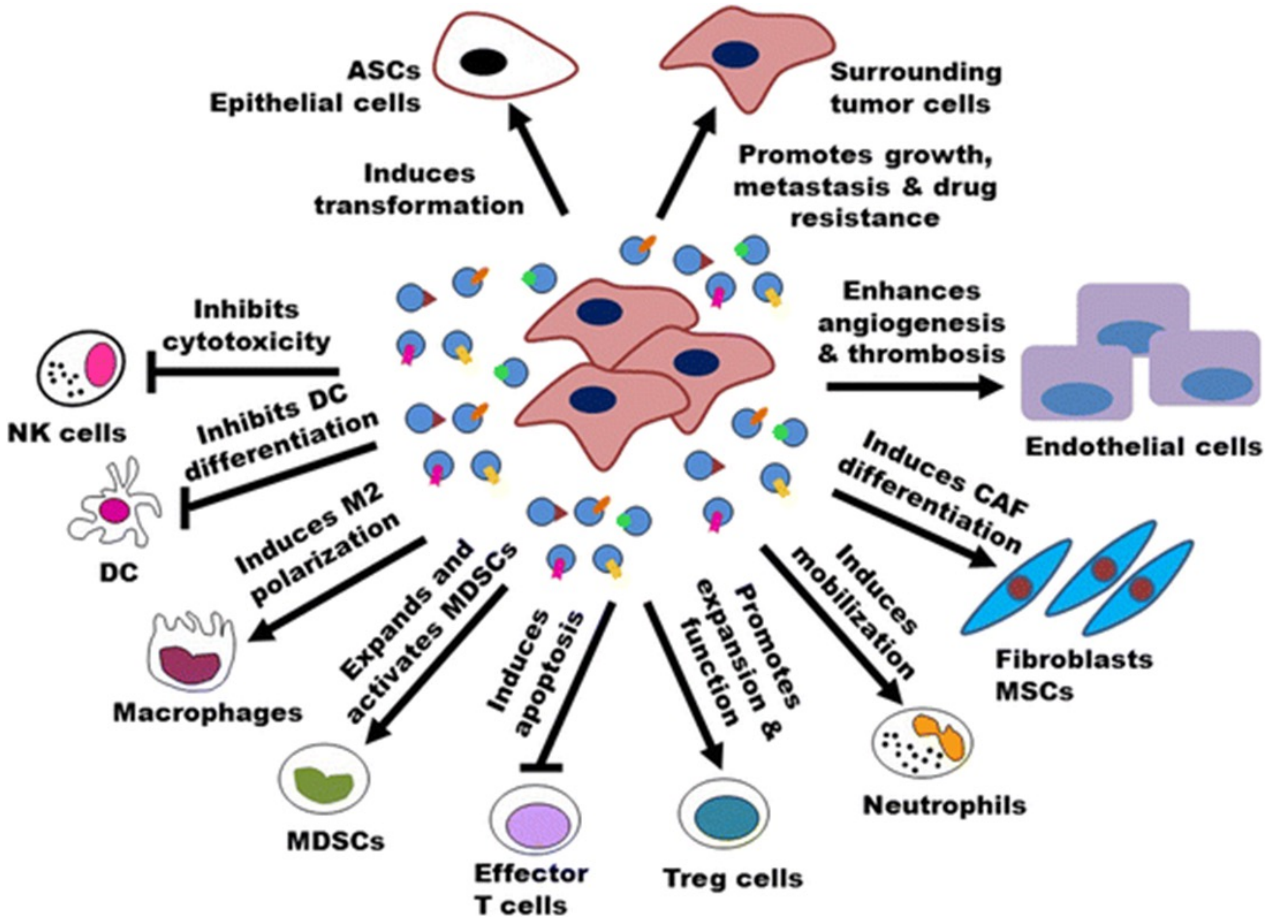


DEBRECENI EGYETEM

Soltész B et al., unpublished

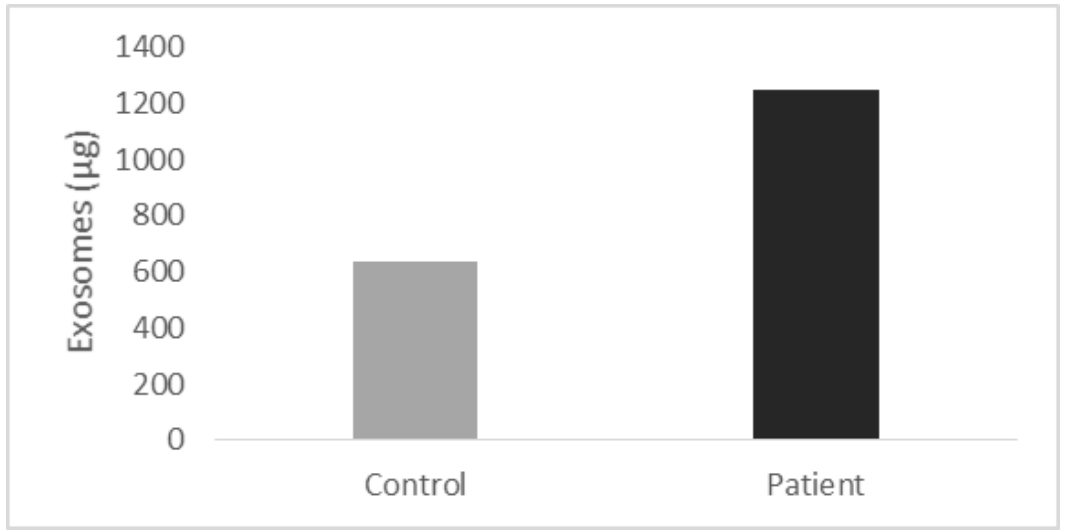
Exosomes in tumours

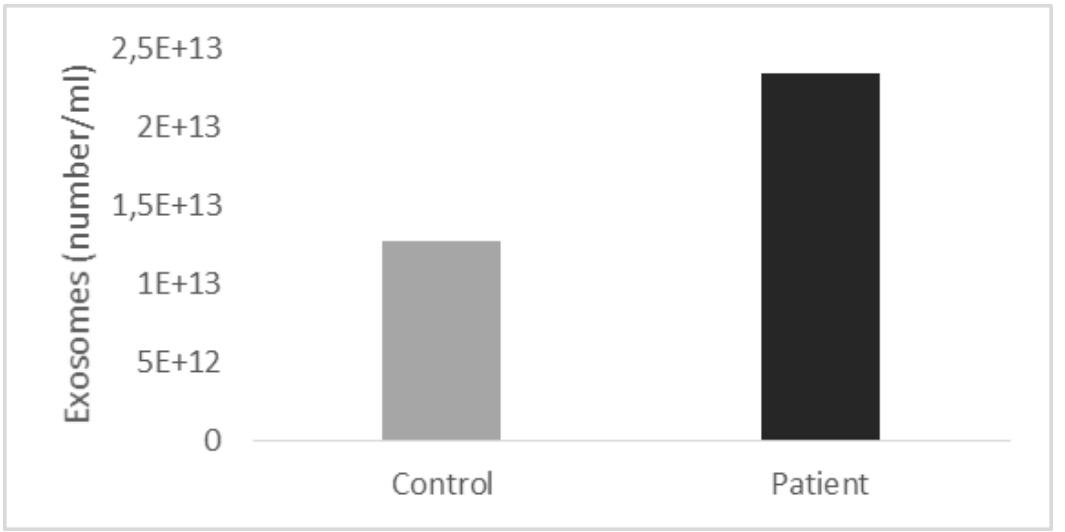
- participate in cancer progression and metastasis
- transfer bioactive molecules between cancer and various cells (local and distant microenvironments)



Zhang et al. J Hematol Oncol. 2015;8:83.

Exosomes in ovarian cancer





DEBRECENI EGYETEM

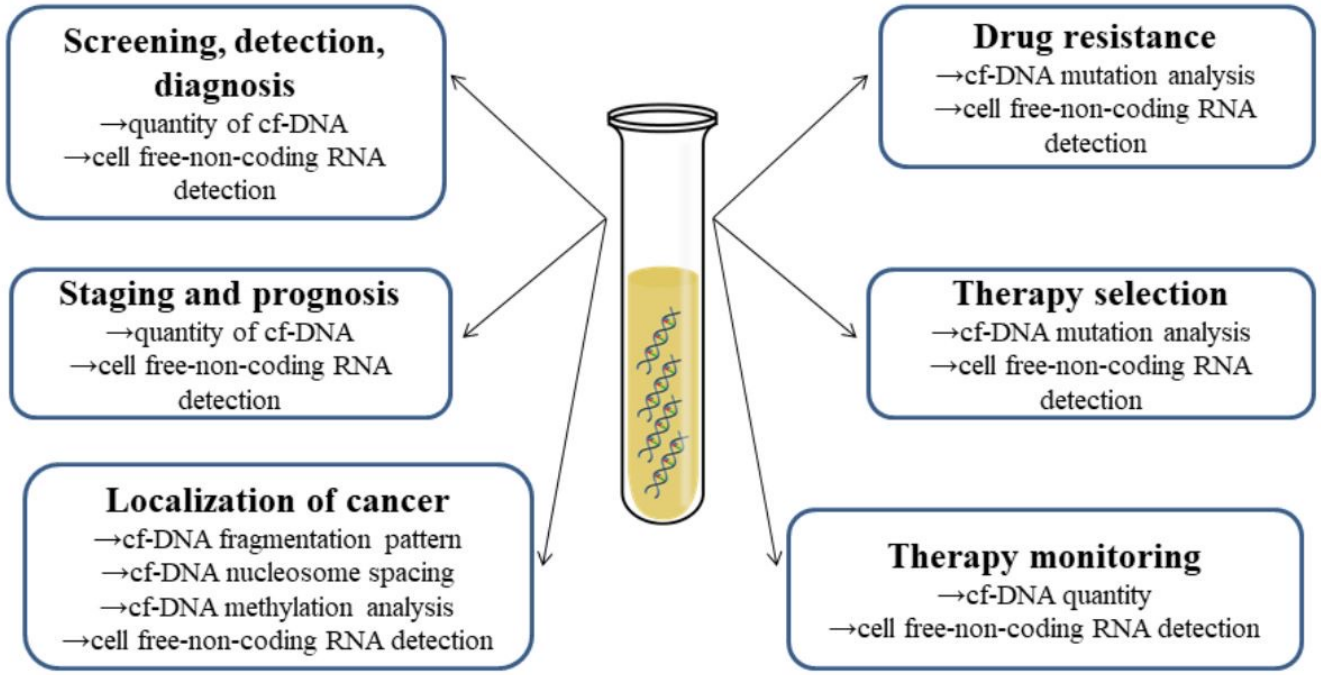
Conclusions

- New possible pathway in the development of ovarian cancer
  - MALAT1 – miR146a – CD24
- *miR125b*, *miR146b, miR200b* and especially, the *miR146a* are promising candidate plasma biomarkers in the diagnosis of OC
- Significant difference in CD24 expression between the OC and C groups (correlation with FIGO classification)
- Higher exosome quantity in OC than in C group

DEBRECENI EGYETEM

Clinical significance of cf-NAs

- Non-coding RNA (e.g. miRNA) expression in cancer cells differs from non-coding RNA expression in healthy cells → promising biomarker candidates
- Cf-NA expression differs in the body fluids of cancer patients and healthy individuals



DEBRECENI EGYETEM

Future plans

- More sensitive detection methods need to be developed
- Understanding the biological role/importance of non-coding RNAs in cell cultures
- Development of multivariate diagnostic tests: combination of several biomarkers

DEBRECENI EGYETEM

ACKNOWLEDGMENT

- Prof. Dr. Bálint Nagy
- Prof. Dr. István Balogh
- Dr. András Penyige
- Dr. Orsolya Biró
- Dr. János Lukács
- Gréta Gombos
- Nikolett Németh
- Dr. Zoárd Krasznai
- Prof. Dr. Róbert Póka

DEBRECENI EGYETEM

Thank you for your attention!

DEBRECENI EGYETEM