Involvement of RNA-Exosome Machinery in the Pathophysiology of Prostate Cancer

Analysis of the Expression of the DIS3 Gene in Different Cell Lines

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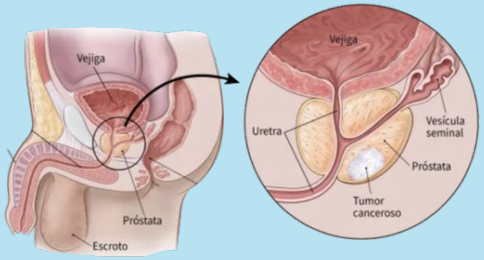
Authors' education level: first year of high school



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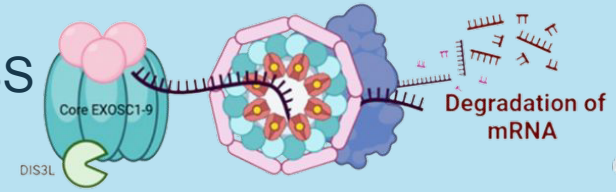

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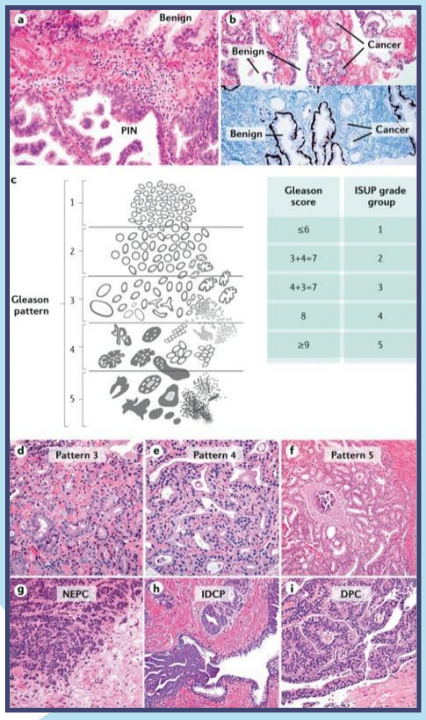
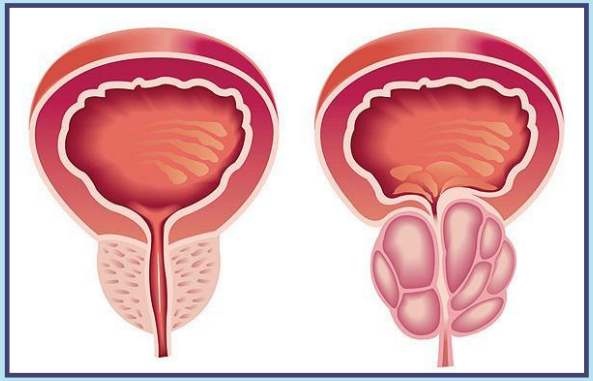
Introduction



Gleason score



Normal prostate

Prostate cancer

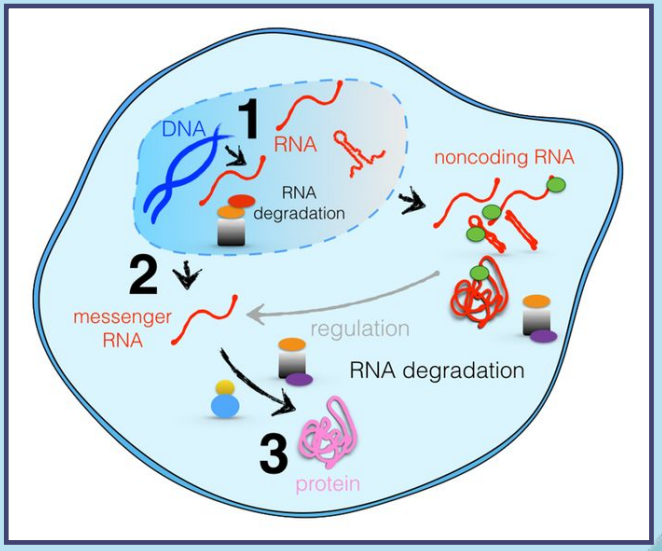
Hypothesis

Prostate cancer is the most common tumor in men in developed countries. It originates in the prostate gland when its cells grow uncontrollably. The Gleason classification allows for assessing its aggressiveness by analyzing cancer cells under a microscope. TNM staging, along with the Gleason score and PSA levels, helps determine the extent of the cancer, and the most appropriate treatment.

The reason for this investigation

Relevance of RNA-Exosome in Prostate Cancer

- Exosome RNA is a cellular machinery that degrades and regulates different types of RNA.
- Its study has gained interest due to its role in diseases such as cancer and the rise of mRNA therapies.
- Understanding its function could improve the diagnosis, prognosis, and treatment of this tumor.



Previous Scientific Investigations: PSA and Diagnosis

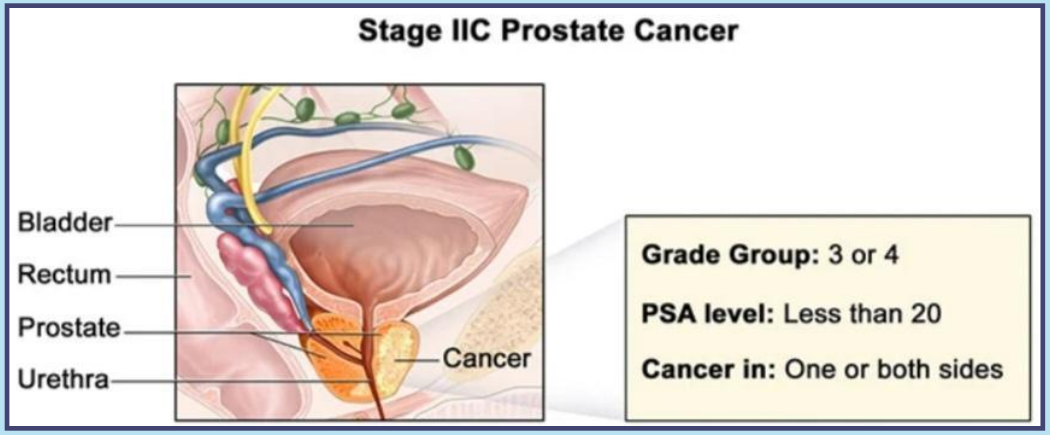
🧪PSA (Prostate-Specific Antigen):

- Discovered in the 1970s, it revolutionized prostate cancer detection.
- An elevated level can indicate cancer, but also other benign conditions.

📉 Limitations of PSA:

Low specificity, due to:

- Benign prostatic hyperplasia (BPH).
- False negatives.
- Prostatitis.



🔍 Complementary markers to PSA:

- Free/total PSA.
- PCA3 (analyzed in urine).
- Multiparametric magnetic resonance imaging (mpMRI).

Molecular Investigation: Exosome RNA and the PABPN1 Gene

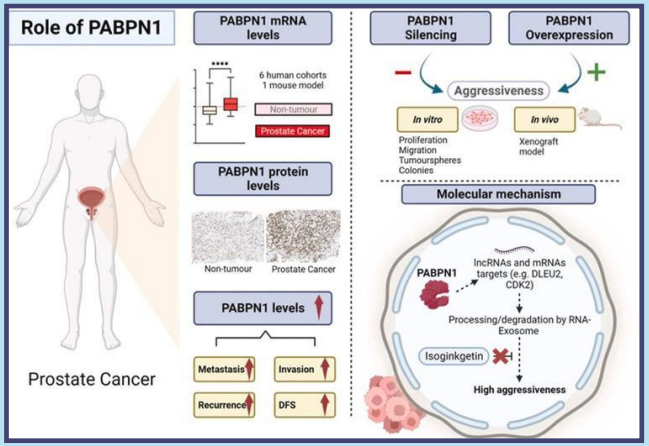
UITM - Caixa Research (VHIO):

In 2023:

- 249 Phase I trials with active recruitment.
- 25 Basket-type trials, which test a drug in different types of cancer with the same molecular alteration.

Study on Exosome RNA and the PABPN1 Gene:

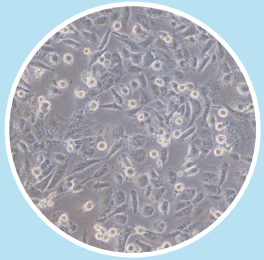
- The PABPN1 gene is overexpressed in tumor cells.
- Its overexpression promotes: Proliferation, Migration, Tumor cell invasion.
- Silencing PABPN1 reduces tumor aggressiveness.



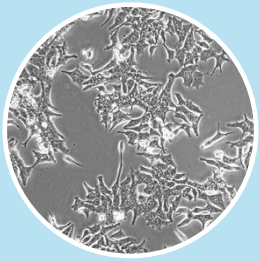
Graphical abstract

Objectives

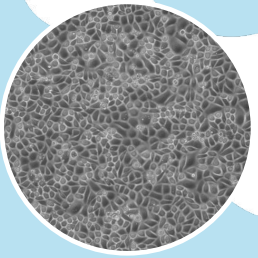
The purpose of this research is to determine how the DIS3 gene behaves in prostate cancer cells (VCaP, Du145, and 22Rv1) as opposed to normal prostate cells (RWPE-1).



Du145



22Rv1



RWPE-1

This study attempts to determine whether lower DIS3 levels are linked to more aggressive cancer by utilizing quantitive PCR to measure the amount of DIS3 expressed in several prostate cancer cell lines by contrasting it to normal cells.

This could help identify the potential of DIS3 as a biomarker or perhaps a means of diagnosis for prostate cancer.

Theoretical Framework

Prostate Cancer Staging and Treatments

Prostate Cancer Staging (TNM + Gleason)

Stage 1 (T1):

- Small, non-palpable tumor.
- Low grade (Gleason ≤ 6).

Stage 2 (T2):

- Larger tumor but still confined.
- Possibly more rapid growth (Gleason 7).

Stage 3 (T3):

- Has spread to nearby tissues .
- Possible local lymph node involvement.

Stage 4 (T4):

- Cancer has metastasized (bones, lungs, etc.).
- More aggressive, with a worse prognosis.

💊 Treatment Options According to Stage

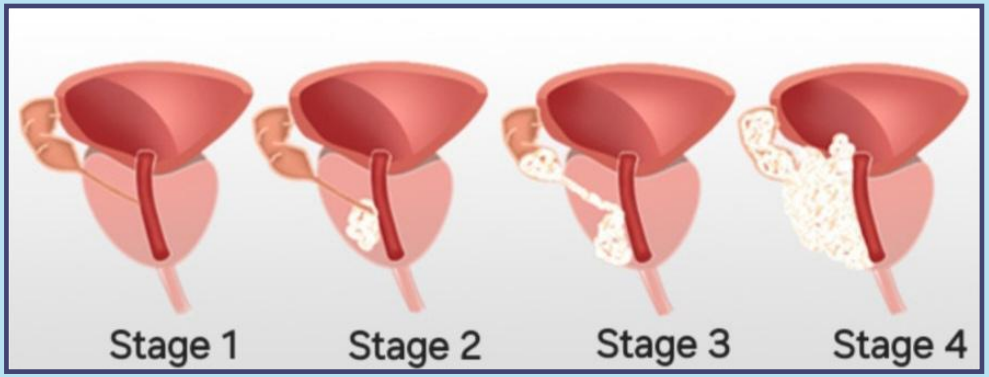
Localized cancer (stages 1 and 2):

- Radical prostatectomy.
- Radiation therapy as support if there is a high risk of relapse.

Advanced or recurrent cancer (stages 3 and 4):

- Androgen deprivation therapy (ADT):
- Surgical castration or drugs (GnRH analogues or antagonists).

Advanced hormonal treatment: Enzalutamide, Abiraterone



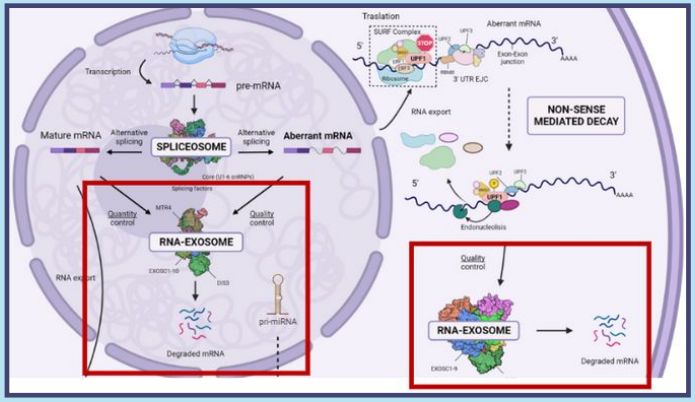
RNA-Exosome, DIS3 Gene, and New Therapeutic Perspectives

Exosomes and RNA in Prostate Cancer

- Exosomes are vesicles that transport RNA (microRNA, lncRNA).
- They influence tumor progression, metastasis, and treatment resistance.

RNA-exosome machinery:

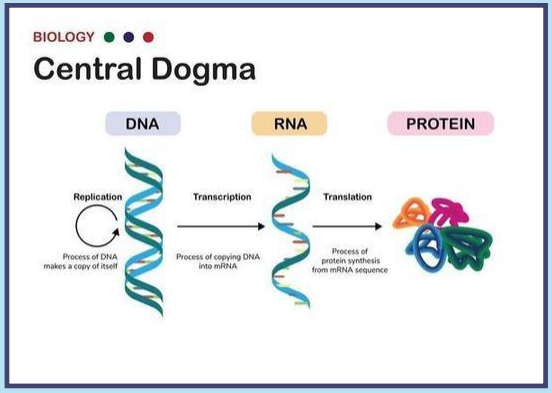
- Cellular complex that eliminates defective or unnecessary RNA.
- Ensures that only functional RNA remains in the cell.



Central Dogma and the Role of the DIS3 Gene

DIS3 Gene: Key ribonuclease of the exosome.

- It regulates the degradation of aberrant RNA.
- In the prostate, it could be a therapeutic target to control tumors.



DNA → RNA → Protein (basic gene expression).

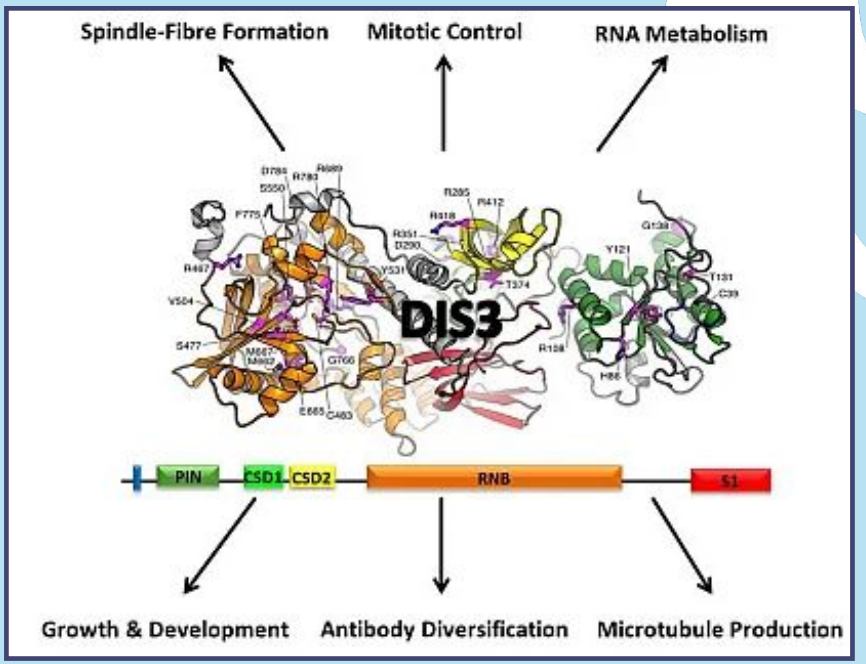
Materials and Methods

Variables of research

- **Independent variable**
  - Prostate cancer cell lines: the different expressions of the study gene DIS3 will be observed in these.

- **Dependent variable**
  - DIS3 gene expression levels: it is the aim of the investigation. Results will be specific to each cell line studied.

- **Control variables**
  - Housekeeping genes: they are those genes that are expressed in a constant way
  - RNA quantity and quality: constant values are set.
  - Conditions of qPCR: the reliability of results obtained is ensured



Materials and Methods

MATERIALS

- **Pellet of cells**
  - Du-145
  - 22Rv1
  - VCaP

  Cell lines
  - RWPE1
  - GAPDH
  - ACTB
  - DIS3

  Genes

- **Trizol**

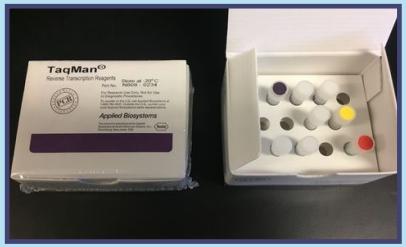
- **Chloroform**

- **Isopropanol**

- **Ethanol 75%**

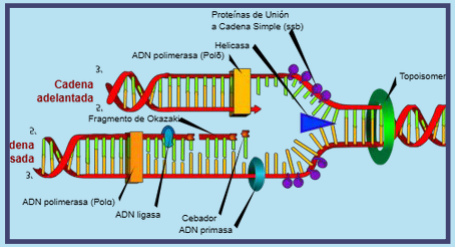
- **H2O free of Rnaasas**

- **Retrotranscription kit**

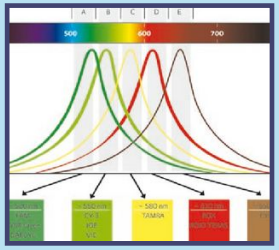


Materials and Methods

- **Primers**



- **PCR SYBR fluorophore**



- **Micropipettes and tips**

- **Cooled centrifuge**

- **Eppendorf tubes (1,5mL)**

- **PCR plates**

- **Nanodrop**

- **Thermoblocks**

- **qPCR thermal cycler**

Materials and Methods

METHODS

- **Collection of samples:** immortalized lines acquired from a commercial house.
- **RNA extraction:**

Materials

- Large box with ice
- Trizol Reagent
- Blue, yellow and white tips
- Chloroform
- Glycogen
- PBS 1x
- Isopropanol
- Eppendorf tubes

	Trizol	Chloroform	Isopropanol
Plate of 12 or 14	600 µl	120 µl	300 µl
Plate of 6	1 ml	200 µl	500 µl

Procedure

- Preparation of the procedure
- Collection with Trizol
- Extraction with Trizol
- Treatment with DNAase

Materials and Methods

METHODS

- **Determination of the concentration and quality of extracted RNA:** it is ensured that such RNA samples are in proper conditions.

	22Rv1	Du145	VCaP	RWPE 1
n1	338,9	819,5	217,2	162,7
n2	259,8	515,1	125,9	134,7
n3	1119,9	468,0	121,5	166,9

- **Retrotranscription from RNA messenger (RNAm) to DNA copy (DNAc):**

Precautions

- Keeping materials in ice
- Preparing an extra tube for the MIX
- Total volume is 11µl (RNA + H2O)

Materials

- MIX 8µl/sample
  - 4µl Buffer
  - 2µl dNTPs (10mM)
  - 1µl Ribolock
  - 1µl Reverse Transcriptase

Procedure

- Add RNA and H2O to the tubes and spin at 4ºC
- Add 1µl primers, spin and incubate 5min. at 65ºC
- Add MIX and resuspend with the pipette
- Leave it 5min at RT.
- Set it 1h at 42ºC
- Set it 5 min at 70ºC

Materials and Methods

METHODS

- **Amplification and quantification by qPCR:** they allow simultaneous amplification and quantification of a DNA fragment, monitoring in real time the generation of the product by the amplification curve.

Materials

Component	Volume
Primer Fw	0.3 µl
Primer Rv	0.3 µl
Máster MIX (SYBR)	5 µl
H_2O_d (autoclaved)	3.4 µl
ADN copy	1 µl (≅ 50 ng/µl minimum quantified in NanoDrop)

Procedure

- Mix qPCR/well:
  - Defrosting samples, primers and curve
  - Turn instrument, computer, instrument lamp and qPCR thermal cycler on. Open Mxpro.
- Prepare a mix:
  - Add Master MIX, H2Od (autoclaved) and primers
  - Mix it with the pipette, give a vortex and a spin
  - Add 19µl of mix to all wells of the same gene with the same tip
  - Vortex to the curve and shake tubes manually
  - Take 1µl of the mix

Materials and Methods

Planning of research

- **Session 1 (06/11/2024) (4 hours):** facilities presentation and introduction to the project.

- **Session 2 (03/12/2024) (4 hours):** description focused on the materials and methods. RNA extraction and reverse transcription.

- **Session 3 (15/01/2025) (4 hours):** quantitative PCR (qPCR).

- **Session 4 (12/02/2025) (4 hours):** analysis and discussion of results obtained. The scientific poster is made.

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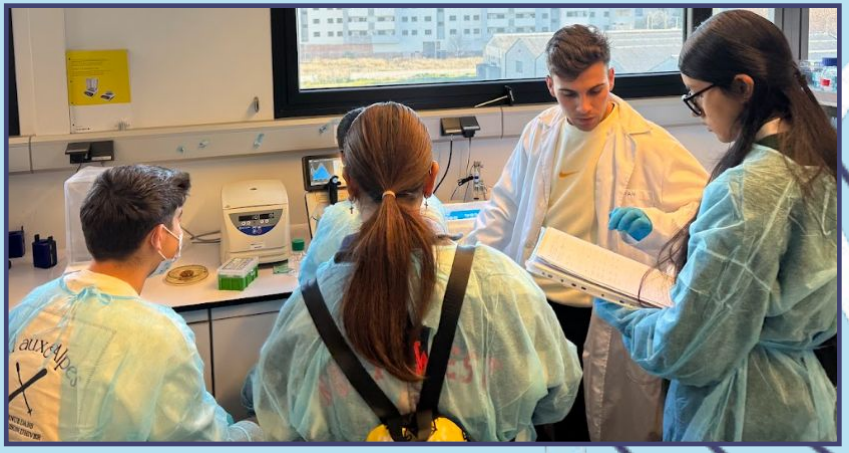
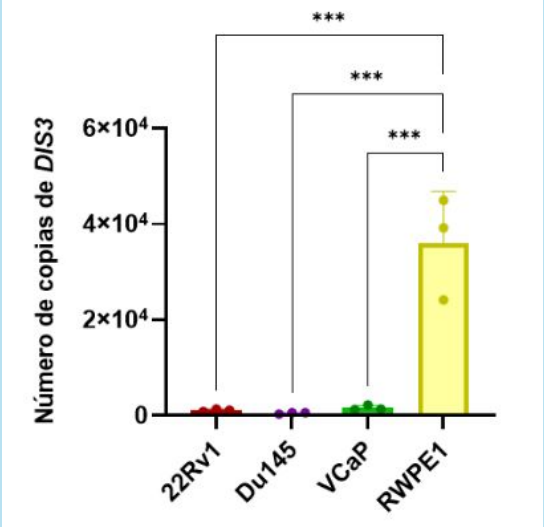
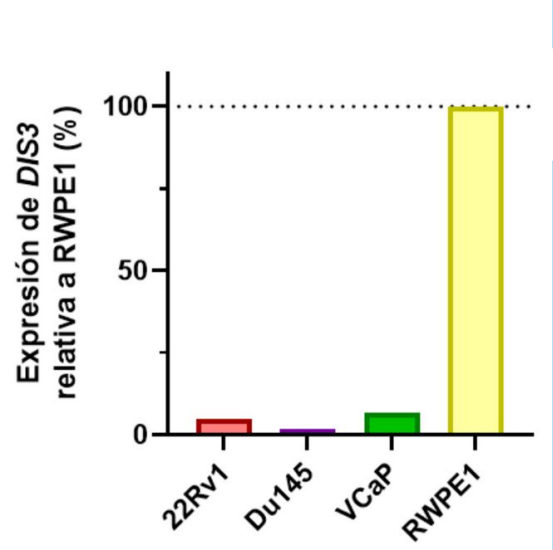
3º



Results & Discussion

Type	ARN (ng/µL)	A260/A280	A260/A230
VCaP n1	217.20	2.11	1.97
VCaP n2	125.90	2.09	1.47
VCaP n3	121.50	2.09	1.45
RWPE1 n1	162.70	2.13	1.98
RWPE1 n2	134.70	2.12	1.98
RWPE1 n3	166.90	2.12	1.94
22Rv1 n1	338.90	2.14	1.81
22Rv1 n2	259.80	2.14	2.06
22Rv1 n3	1119.90	2.13	2.09
DU145 n1	819.50	2.14	2.14
DU145 n2	515.10	2.12	1.84
DU145 n3	468.00	2.10	1.90





We observed a significant decrease of DIS3 in the tumour lines VCaP, DU145 and 22Rv1 compared to the non-tumour line RWPE1. For example, DU145 showed only 1% of the expression observed in RWPE1.

This reduction is consistent with studies in myeloma and leukaemia, where DIS3 limits malignant proliferation.

Functional implications DIS3 is key to the degradation of defective RNA by the exosome. Its loss could allow the accumulation of abnormal RNA, thereby promoting tumour transformation and progression.

Conclusions

- DIS3 is expressed at different levels across cell lines.
- the Ct values for DIS3 are higher (27-29) in 22Rv1, Du145, and VCaP (the cancer cells), meaning there is a lower expression in these prostate cancer cell lines
- On the other hand, the Ct values for DIS3 are lower (22-23) in RWPE, a normal cell line, meaning higher expression in normal cells compared to cancerous ones

What does this mean?

The fact that normal cells have much higher DIS3 expression than cancer cells suggests that DIS3 could play a protective role, possibly keeping cell growth and gene activity in check.

In cancer cells, where DIS3 expression is low, this control might be lost, allowing the cells to grow uncontrollably.

If confirmed, DIS3 could become a useful marker for diagnosing prostate cancer or even a target for future treatments.

Potential Treatment!

Acknowledgments

We deeply thank:

- Antonio Prats-Escribano and Lourdes de la Mata Sáez for their invaluable guidance and advice throughout this research.

- The IMIBIC team for their technical support and for providing us with the necessary resources to carry out this study.

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- We acknowledge the collaboration of our colleagues, whose contributions were fundamental to the interpretation of the results

- Finally, we truly appreciate everyone present for allowing us the opportunity to present our findings at an international level

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Thank you!

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