ALTERATION OF THE EXPRESSION OF REGULATORY COMPONENTS OF ARN METABOLISM IN PROSTATE CANCER

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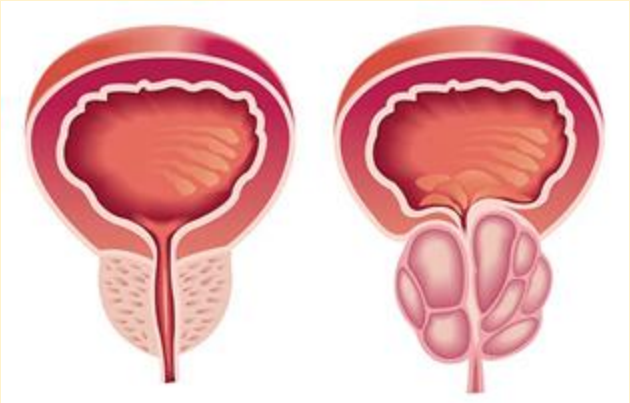
1.- INTRODUCTION

Prostate cancer represents the tumor pathology with the highest incidence in the male population of developed countries. Currently, the main therapy consists of blocking androgen signaling. However, a large number of patients become resistant to it, progressing to the most aggressive type of this disease, castration-resistant prostate cancer (CRPC). Despite the recent development of androgen receptor (AR) signaling inhibitors, CRPC remains lethal, making the development of new global and effective diagnostic, prognostic and/or therapeutic targets useful for the management of prostate cancer crucial.

Thus, the possible deregulation of some of these control machineries (e.g. nonsense-mediated decay) in prostate cancer may be associated with the onset, progression, aggressiveness of these tumors.



2.- OBJECTIVES

To analyze the expression of markers of aggressiveness in prostate cancer and regulatory components of RNA metabolism in response to treatment of tumor cells with pharmacological inhibitors.

Meeting Abstract: 2017 Genitourinary Cancers Symposium

FREE ACCESS | Prostate Cancer—Advanced Disease | March 29, 2017

Targeting reciprocal feedback inhibition: Apalutamide and everolimus in patients with metastatic castration-resistant prostate cancer (mCRPC).

Authors: Dana E. Rathkopf, Susan F. Slovin, Michael J. Morris, Daniel Costin Danila, Anthony Delacruz, Gregory Shelkey, Mia DeNunzio, Brigit McLaughlin, and Howard I. Scher | AUTHORS INFO & AFFILIATIONS

ONC201/TIC10 enhances durability of mTOR inhibitor everolimus in metastatic ER+ breast cancer

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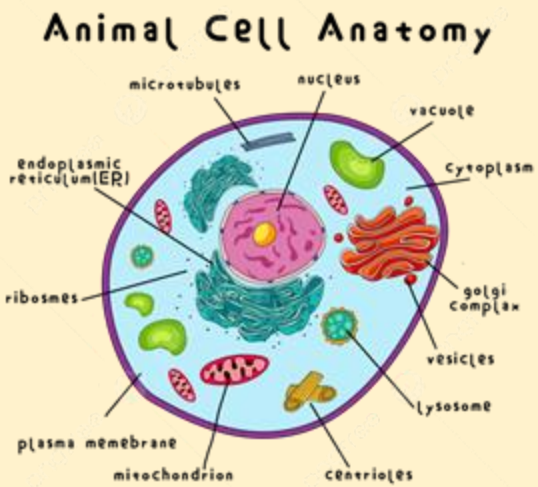
3.- THEORETICAL FOUNDATIONS.

The human organism is made up of more than 200 different types of cells, which are the basic functional units and whose internal structure is complex.

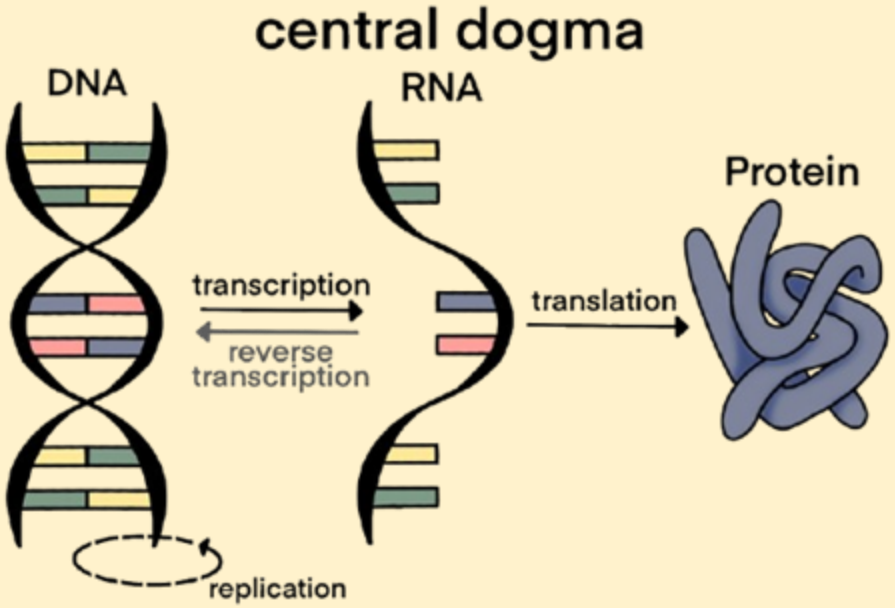
One of its components (organelles), which is important to understand the process of cancer, is the nucleus, where the DNA is located and the genes that control cell reproduction are found.



DNA, a nucleic acid, contains the fundamental genetic instructions for the development of living beings.

FOUNDATIONS

The information in DNA is not used directly; it follows a three-step pathway:



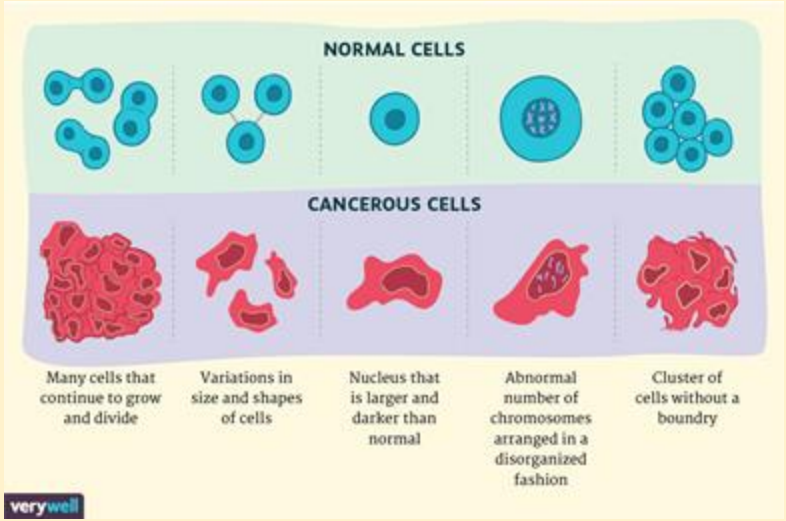
FOUNDATIONS

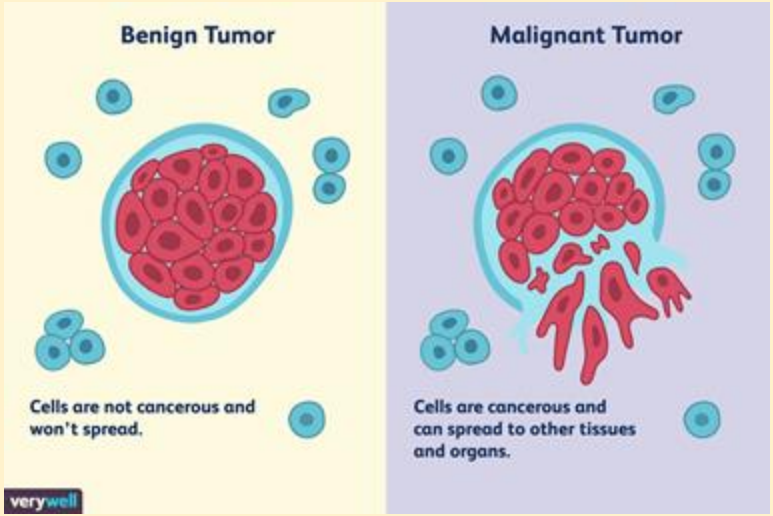
Under normal conditions, cells grow and divide to form new ones and this process is called cell division and is carried out in an orderly and controlled manner.

However, in cancer, cells grow uncontrollably, forming masses called tumors. Some of them can even travel to other parts of the body, which is more dangerous.

There are two types of tumors:

→ **Benign.**
→ **Malignant**.



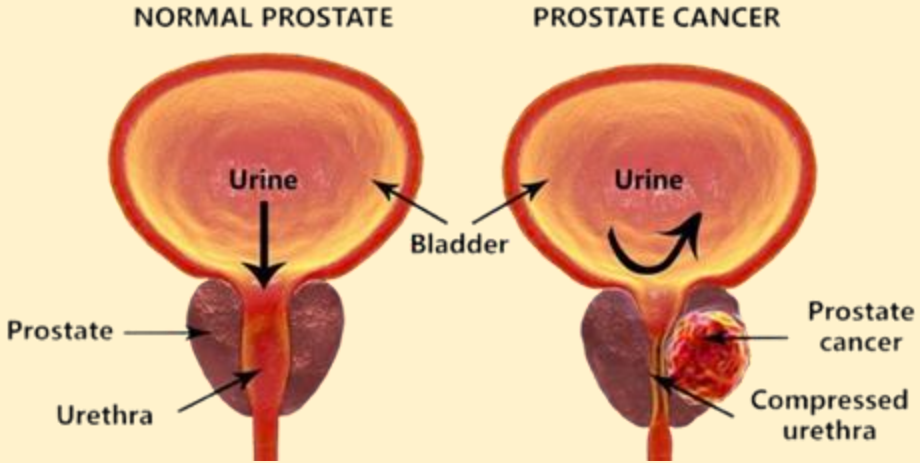


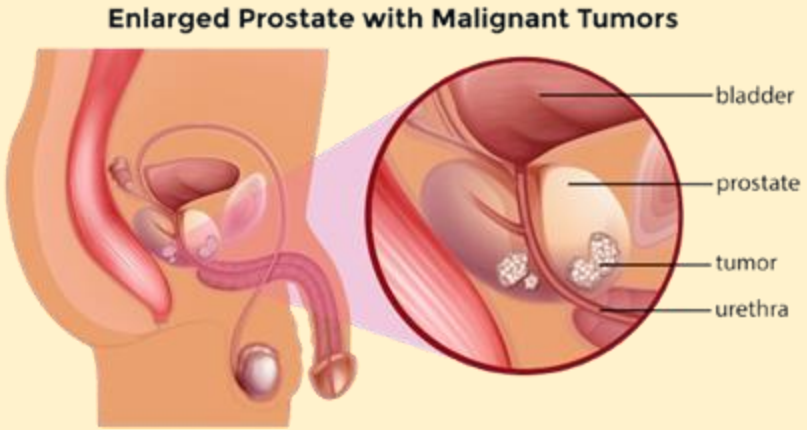
FOUNDATIONS

PROSTATE CANCER.

The prostate is a small gland that only men have. It is just below the bladder and surrounds part of the urethra, which is the tube through which urine passes.

Prostate cancer occurs when some cells in this gland begin to grow uncontrollably. If detected early, it is treatable.



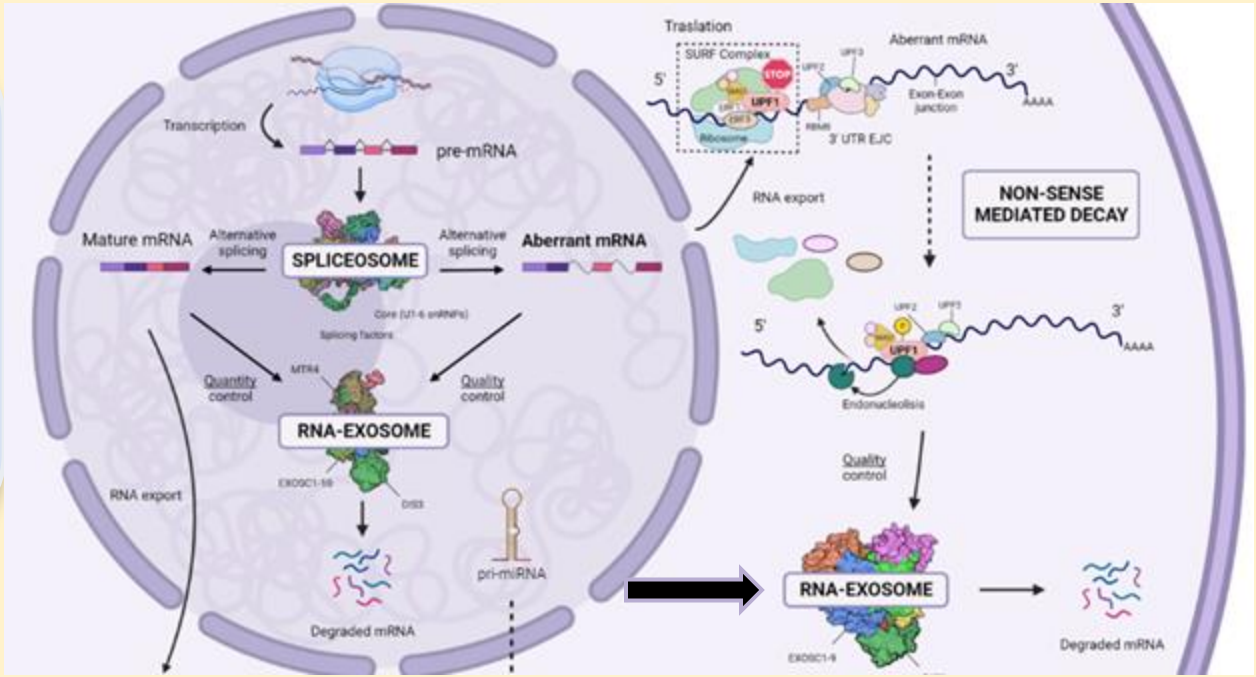


FOUNDATIONS

HOW TO CONTROL IT?

Inside cells, there are systems that make sure that the instructions from DNA, which are passed to RNA, are used correctly to create proteins. If something goes wrong with the information in the RNA, defective proteins could be produced, which sometimes contributes to diseases such as cancer.

This system is like a "garbage disposal" for RNA. When the cell detects that an RNA is not working properly or is not needed, the exosome breaks it down and eliminates it.



4.- MATERIALS AND METHODS

Variables

- **Independent variable:** Everolimus treatment.
- **Dependent variable:** CNOT4 and RUVBL1 gene expression (qPCR).

REAGENTS (trizol, isopropanol, chloroform, etc.)

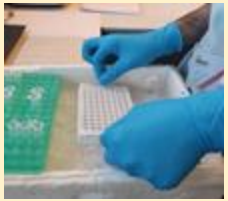
RETROTRANSCRIPTION KIT (primers, master mix)

REFRIGERATED CENTRIFUGE

TERMOBLOCKS

NANODROP

EPENDORF TUBES, MICROPIPETTES, PCR TIPS AND PCR PLATES



qPCR THERMOCYCLER

EXPERIMENTAL DESIGN

1.- mRNA extraction

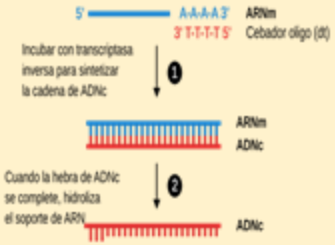
ARN EXTRACTION HOOD

DNAsa

QUANTIFICACION

2.- mRNA to cDNA retrotranscription

RETROTRANSCRIPTION



qPCR LOAD

3.- Transcript quantification by qPCR

qPCR MACHINE. Thermocycler.

5.- RESULTS

1. RNA QUALITY

Table 1 shows the results obtained using the Nanodrop. The concentration level of the samples (necessary to perform RNA RT calculations) and their purity were consulted to determine whether they were valid. Their level had to be around 2 and, as can be seen, they all complied. Discard protein contamination (260-280) around 3+2 does not contaminate. 260-230 around 2 does not contaminate with organic solvents.

Muestra	ng/µl	A260/A280	A260/A230	A260	A280
1	169,5	2,12	2,07	4,24	2,00
2	425,2	2,15	1,68	10,63	4,96
3	291,8	2,13	2,06	7,29	3,42
4	393,9	2,14	1,67	9,85	4,59
5	1136,9	2,13	2,13	28,42	13,37
6	771,2	2,12	2,11	19,28	9,10

RNA concentration and purity ratios of samples

RESULTS

2. QUANTIFICATION BY qPCR

Table 2: Results obtained: Cycles obtained in qPCR

	DMSO			Everolimus		
	n1	n2	n3	n1	n2	n3
ACTB	326266,17	364004,44	445677,11	289783,42	348872,56	361783,41
CNOT4	10444,38	11332,83	13076,89	4400,93	5042,13	5165,26
RUVBL1	2757,14	2860,68	3084,84	4619,20	5252,51	5437,22

DMSO: *control.*
Everolimus: *treatment. Potent inhibitor of tumor cell growth and proliferation.*
ACTB: *gene used as control that does not vary its expression at that temperature.*
CNOT4 and RUVBK1: *genes.*

Table 3 shows the expression levels of the different genes with respect to temperature.
n1,n2,n3 refers to the identical biological replicates..

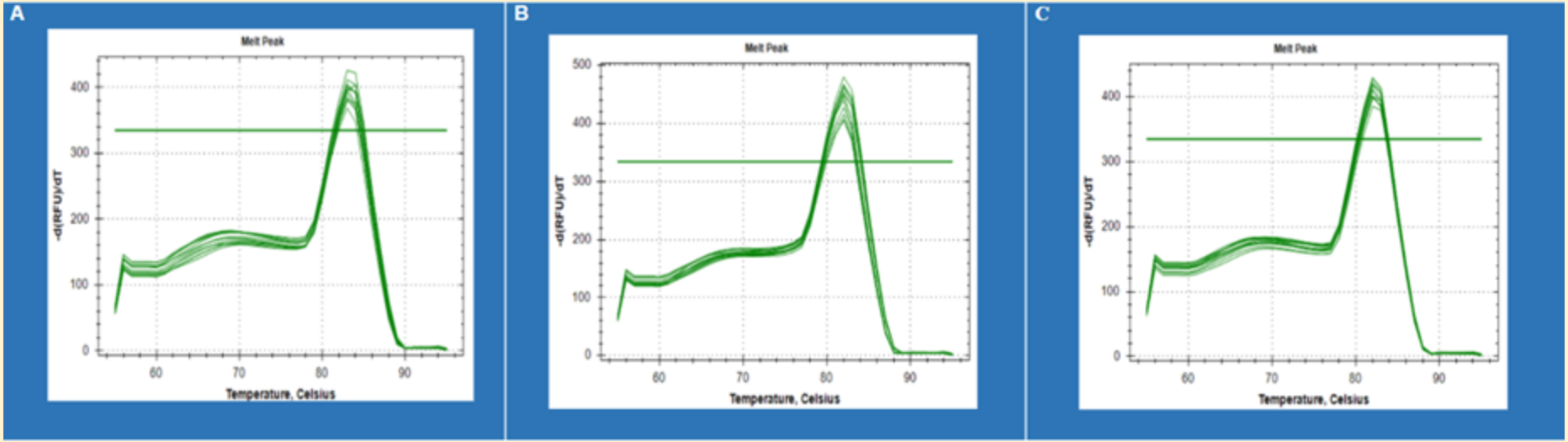
Table 3: Gene expression levels in percentage with respect to temperature (%).

	DMSO			Everolimus		
	n1	n2	n3	n1	n2	n3
ACTB	17,85	17,42	17,25	18,05	17,61	17,75
	17,74	17,91	17,49	17,87	17,8	17,56
CNOT4	24,21	24,1	24	25,49	25,32	25,35
	24,35	24,22	23,9	25,6	25,37	25,27
RUVBL1	26,29	26,15	26,02	25,4	25,27	25,19
	26,17	26,2	26,11	25,55	25,3	25,28

RESULTS

Each graph represents a gene

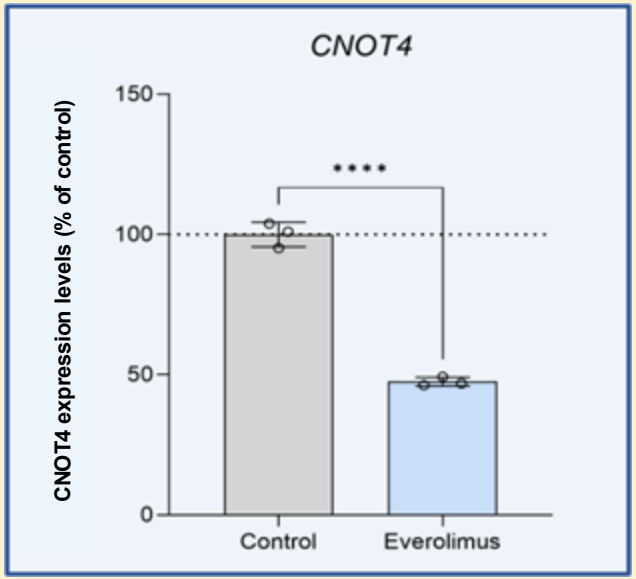
3. MELTING GRAPHS



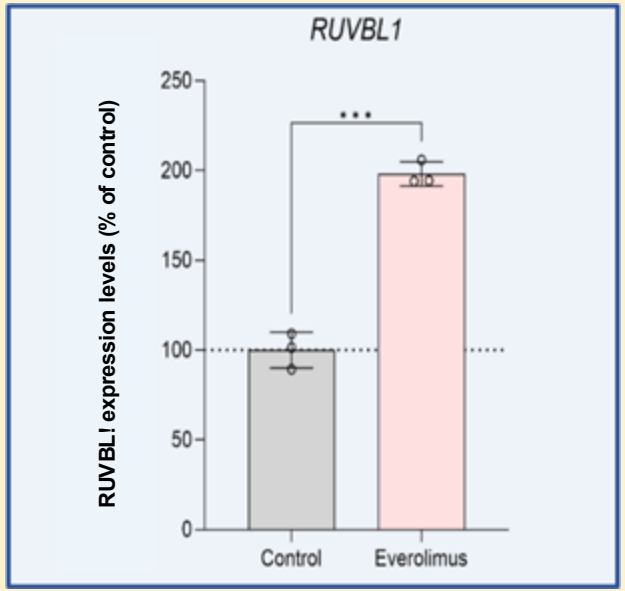
Graph 1: Melting curves in genes studied. Temperature in degrees Celsius is represented with respect to relative fluorescence unit. A) ACTB gene. B) CNOT4 gene. C) RUVBL1 gene.

RESULTS

4. GENE EXPRESSION



Graph 2: CNOT4 gene expression levels in DMSO conditions (control) and in Everolimus treatment. The measurement error (error bar) of the three measurements is indicated.



Graph 3: RUVBL1 gene expression levels under DMSO conditions (control) and Everolimus treatment. The measurement error (error bar) of the three measurements is indicated.

RESULTS

TREATMENT OF THE RESULTS

To compare the expression levels of CNOT4 and RUVBL1 genes between the two conditions (control and Everolimus), the following tests were performed

- Normality test using the **Shapiro-Wilk test**.
- After demonstrating that the data sets follow a normal distribution, a **Student's t-test** was performed for each of the two genes

6.- CONCLUSIONS

- Everolimus treatment decreases the expression of CNOT4 in prostate cancer lines, while, on the other hand, this treatment increases the expression of the RUVBL1 gene.
- In conclusion, Everolimus treatment in prostate cancer could modulate NMD expression and function, although it would be necessary to explore the different mechanisms by which the effect of NMD and its relationship with prostate cancer could be altered.

7.- ACKNOWLEDGMENTS.

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To the Junta de Andalucía and to the project of the Ministry of Science, Innovation and Universities with code: PID2022-1381850B-100.

THANK YOU FOR YOUR ATTENTION

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