DETERMINATION OF EXPRESSION SIGNATURE AND PROPORTION OF mtDNA IN PLASMA FRACTIONS IN PATIENTS WITH RENAL CELL CARCINOMA.

Elena Arance-Criado[1], Fernando Vázquez-Alonso, Mª Yarmila García-Iglesias[3], Rocío López-Cintas[3], Sara Martín-Esteban[4], Ginesa López-Torres[5], Ana Isabel Cortés-Valverde[6], María Jesús Álvarez Cubero[1,7] and Luis Javier Martínez-Gonzalez[2*]

[1]GENYO. Centre for Genomics and Oncological Research. Pfizer / University of Granada / Andalusian Regional Government.
[2]Urology Department, Hospital Virgen de las Nieves. Av. De las Fuerzas Armadas 2, 18014, Granada, Spain.
[3]Gran Capitán Health Center. Calle Gran Capitán, 10, 18002 Granada. [4]Peligros Health Center. Calle Valencia, s/n, 18210 Peligros, Granada.
[5]Salvador Caballero Health Center. Calle Dr. Azpitarte, 6, 18012 Granada. [6]Casería Montijo Health Center. Carr. de Jaén, s/n, 18013 Granada.
[7]Department of Biochemistry and Molecular Biology III and Inmunology, Faculty of Medicine, University of Granada. Av. de la Investigación,11, 18016, Granada, Spain.

INTRODUCTION

Renal cell carcinoma (RCC) is the third most common urologic malignancy[1], and its incidence is increasing globally. Currently, the main issue is the high stage in diagnosis of this disease. This aspect reinforces the need of identifying novel predictive biomarkers for diagnosis, progression and prognosis of RCC[2].

mtDNA exists as a circular, double-stranded nucleic acid with a high copy number. It is known the susceptibility of mtDNA to oxidative stress and mutation[3] and there are some studies that found the relationship between mtDNA copy number (mtDNAcn) and risk of developing several cancer[4,5].

In this study the main objective is to determine the concentration of exosomal mtDNA present in plasma fractions of controls and patients as a potencial non-invasive biomarker of RCC in liquid biopsy.

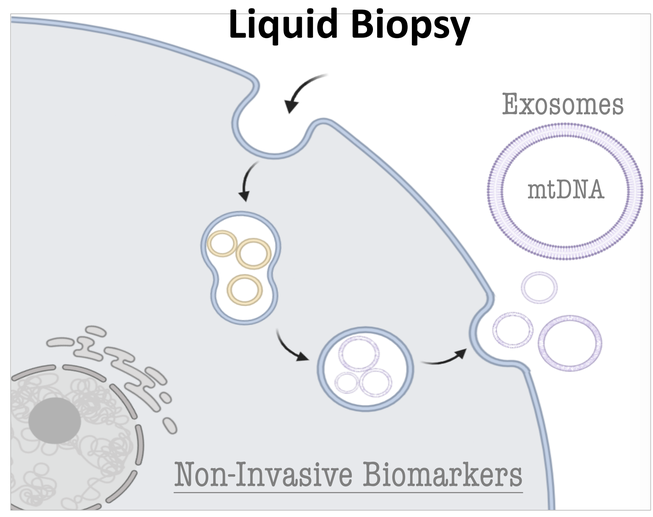
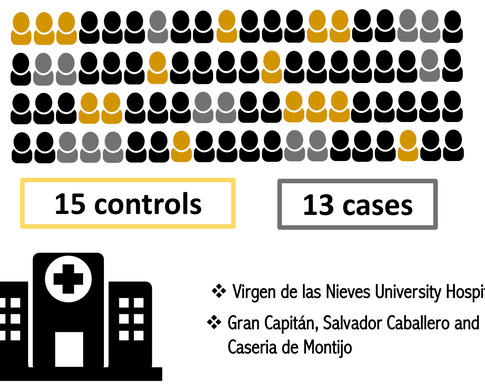


Figure 1: Diagram of the formation of exosomes and emitted vesicles .

MATERIALS AND METHODS

Samples and patients



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Isolation of exosomes

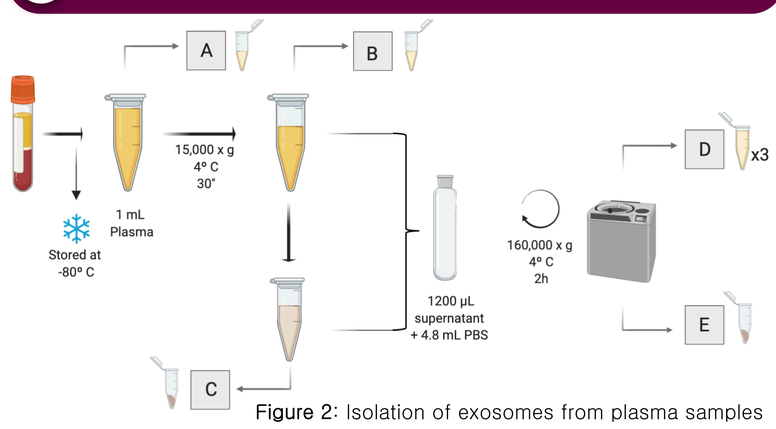
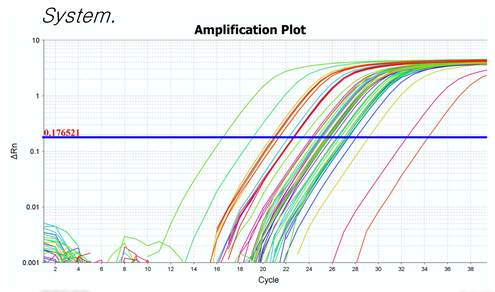


Figure 2: Isolation of exosomes from plasma samples

qPCR

Quantification of mtDNA was performed by *QuantStudioTM 12K Flex Real-Time PCR System.*



HV1 Mitochondrial hypervariable region
CYB Apocytochrome B of complex III
HBB Beta nuclear hemoglobin

RESULTS AND DISCUSION

After making a statistical analysis of the samples to each of the study phases, we observed that in phase F appears the only significant data regarding the difference in DNA concentration between cases and controls (Figure 3).

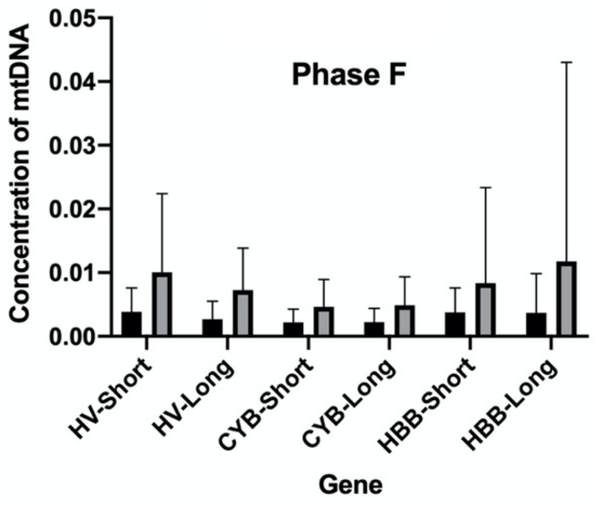


Figure 3: DNA concentration of each gene fragment of controls and patients. The black bars represent the group of the controls, and the grey ones the group of the patients.

In that fraction, with a higher content of exosomal mtDNA, p value shows statistically significant differences in mitochondrial genes HV long and CYB long. While the nuclear gene (both short and long) shows high p values, short fragments for the mitochondrial genes (HV short and HV long) show interesting p values even though they are not significant (Table A3).

It is observed a little increase in the mitochondrial long genes of this study (HV and CYB), due to the protective role of the lipid bilayer of the exosomes (Figure A1).

Gene	P value	Mean of controls	Mean of cases
HV-Short	0,074871	0,003839	0,01005
HV-Long	**0,021676**	0,002671	0,007255
CYB-Short	0,058973	0,002189	0,004655
CYB-Long	**0,049960**	0,002233	0,004892
HBB-Short	0,263638	0,003772	0,008355
HBB-Long	0,410166	0,003674	0,01176

Table 1: Means of each gene fragment of controls and cases in each of the phase F. P value regarding the difference in DNA concentration between cases and controls applying a t-test (t-student).

CONCLUSION

This study allowed us to analyze the fragment size distribution pattern of different regions of interest in each plasma fraction, and we have confirmed the high mtDNA content in exosomes as a powerful biomarker. Therefore, application of liquid biopsy in the clinical scenario is a promising non-invasive technique for prediction, diagnosis and monitoring of cancer treatment. We affirm that it would be quite interesting to study how the amount of mitochondrial DNA varies in controls and patients with RCC in fractions rich in exosomal content, allowing the preservation of mtDNA thanks to its lipid bilayer structure.

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6. Figure 1 and 2 created with Biorender.com