MIRNA DETECTION FOR NON-SMALL CELL LUNG CANCER DIAGNOSIS

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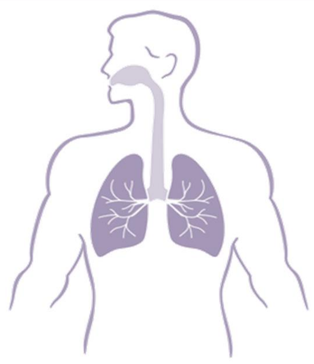
Introduction

- Lung cancer (LC) is the leading cause of cancer-related death worldwide, because of the late diagnosis;
- Circulating miRNAs have been investigated as biomarkers for NSCLC in blood, since they are detectable;



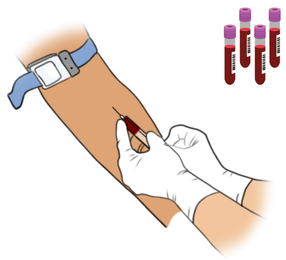
- Peripheral blood mononuclear cells (PBMC's) miRNA profile can be related to NSCLC stages and further diagnosis;
- Molecular beacons (MB) are oligonucleotides comprising a stem-loop structural configuration enabling detection throughout fluorescence.

Aims

- Determine the expression of miRNA-155 in PBMC´s samples of NSCLC patients at different stages.
- Design and biophysical characterization of miRNA-155 MB and *in situ* MB-synthetic miRNA-155 detection.

Methods

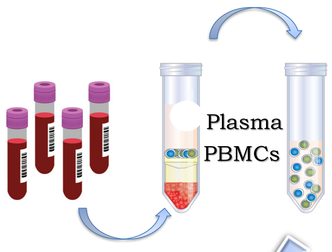
miRNA-155 Profiling



Healthy individuals

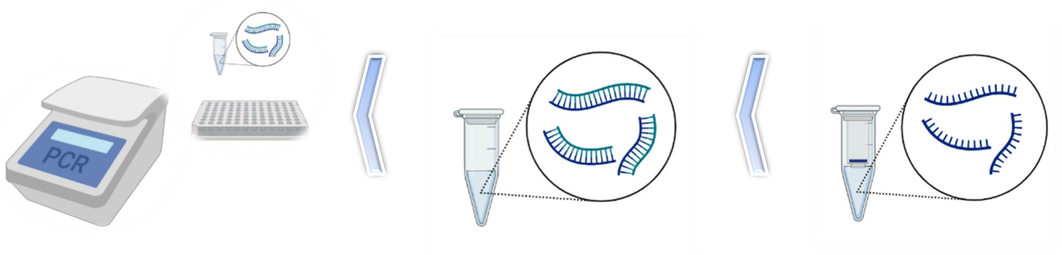
Patients with LC at **different stages**

Groups were established based on the clinical information.



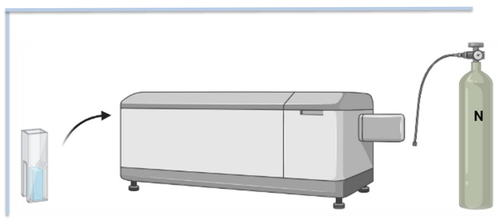
Highly purify PBMCs samples

RT-qPCR assay | **cDNA synthesis** | **RNA extraction**

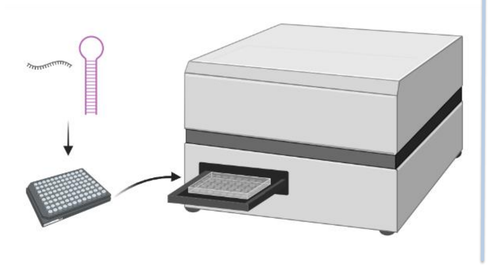


Biophysical characterization and in situ detection

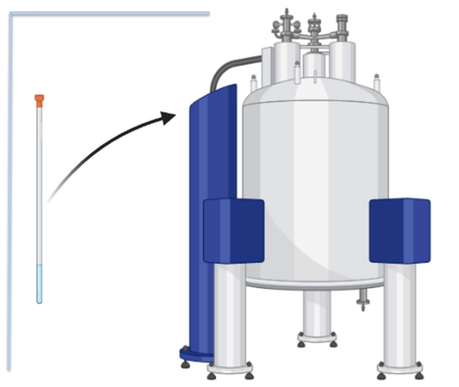
Circular Dichroism



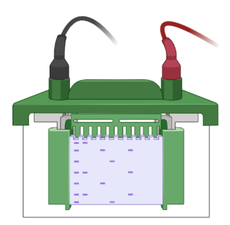
FRET-melting



NMR



Electrophoresis



Results

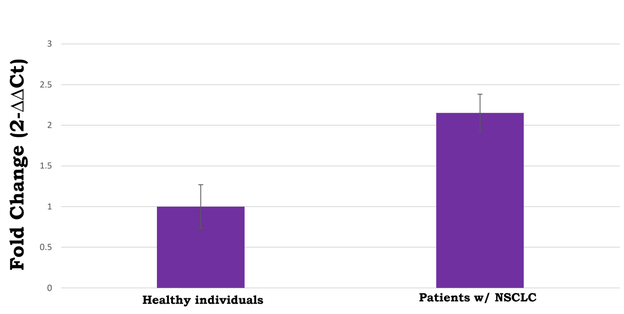


Fig.1 – miRNA-155-3p relative expression in PBMCs of NSCLC patients (n=23) versus healthy individuals (n=10) by RT- qPCR. NSCLC miRNA expression calculated relatively to a healthy control expression value of 1 (mean ± SE. *P<0.05).

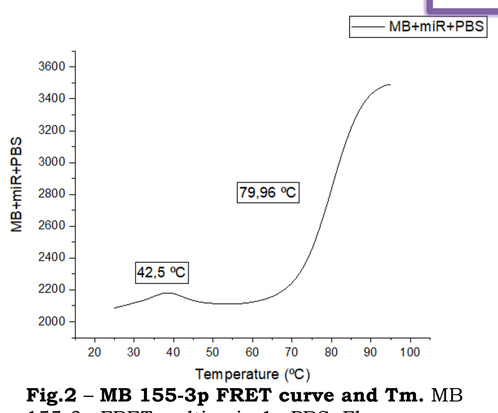


Fig.2 – MB 155-3p FRET curve and Tm. MB 155-3p FRET melting in 1× PBS. Fluorescence was monitored from 25ºC to 95ºC.

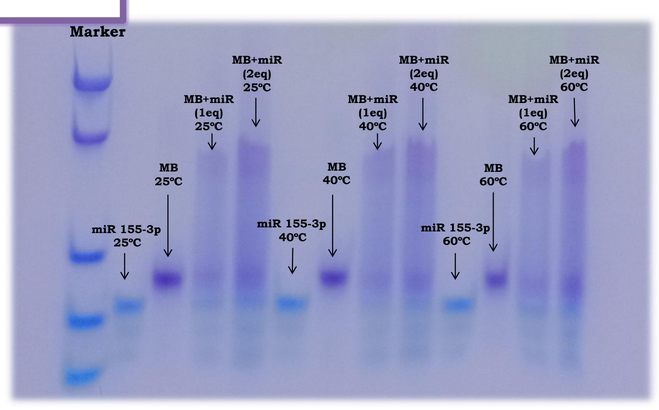


Fig.3 – MB 155-3p hybridization SDS-PAGE electrophoresis gel.

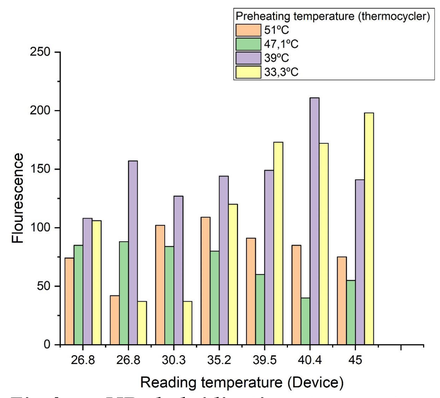


Fig.4 – MB hybridization temperature screening assays. Fluorescence data was acquired in Plate reader 1 and got basal fluorescence discounted.

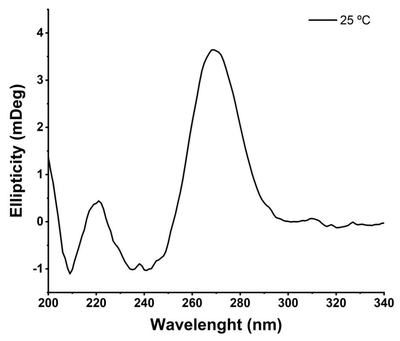


Fig.5 – MB 155-3p CD spectrum in PBS. CD measurements were obtained at 25ºC in a 200-340 nm range.

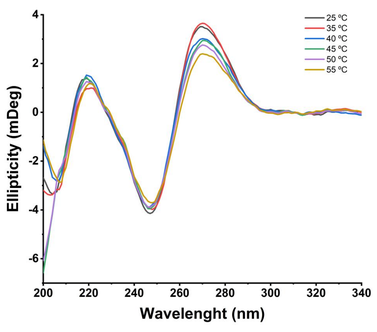


Fig.6 – MB 155-3p CD spectra at different temperatures.

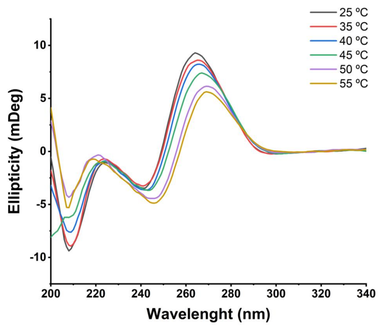


Fig.7 – MB 155-3p with synthetic miRNA-155-3p. CD spectra at different temperatures.

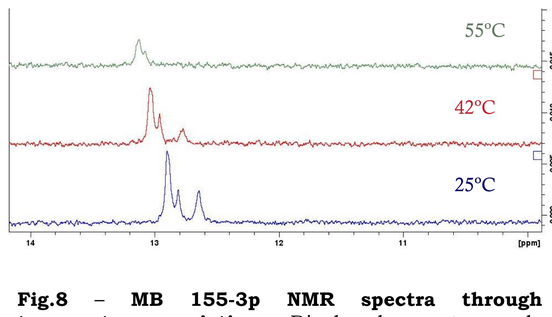


Fig.8 – MB 155-3p NMR spectra through temperature variation. Displayed spectra only comprises the stem region there the MB specific signals are present.

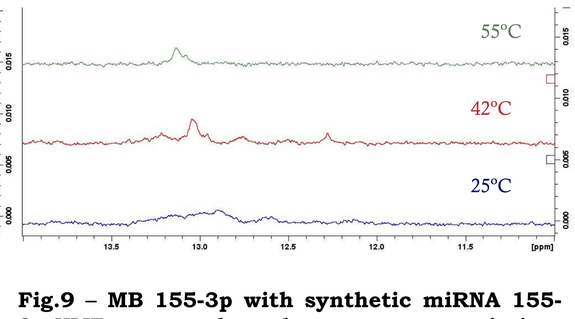


Fig.9 – MB 155-3p with synthetic miRNA 155-3p NMR spectra through temperature variation. Displayed spectra only comprises the stem region there the MB specific signals are present.

Conclusions

- The results revealed up-regulation of miRNA-155-3p in LC patients;
- The characterization of the MB with the synthetic miRNA-155-3p confirmed the specificity of MB as a probe for detection.

Future remarks

- Quantification of the miRNA-155 in plasma samples;
- Development of the to detect and to quantify the miRNA-155-3p in blood samples;
- Increasing the cohort of sample patients.

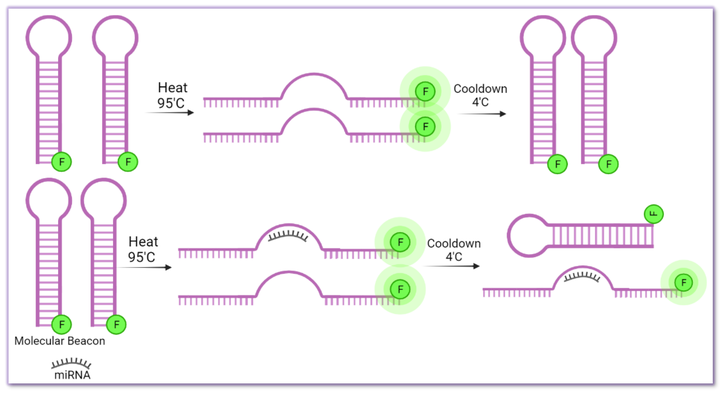


Fig.10 – Representation of the functioning of the MB with or without miRNA-155.

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