Microfluidic Assay Based on G-Quadruplex Aptamer for Nucleolin Detection in Prostate Cancer

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Introduction

Prostate Cancer

2nd most common cancer in men worldwide

In Portugal, most prevalent cancer
1 in 8 men will be diagnosed

Nucleolin (NCL) is protein essential for cancer cell survival

Overexpressed in surface of Prostate Cancer Cells

Binds specifically to DNA G-quadruplex (G4)

Methodology

G-quadruplex Characterization

• Circular Dichroism (CD)
• Nuclear Magnetic Resonance (NMR)
• Fluorometric Assays
• In vitro assays

ELISA immunoassay

Microfluidic experiments

PDMS microfluidic detection system of NCL based on G4 aptamer

• Low reagent consumption
• Simple and Short assay time
• High throughput analysis
• Potential for multiplexing

Results

Fig. 1 – CD titration of AS1411-N5 (A), quantification of target-beacon affinity, through the determination of dissociation constant (Kd) by fluorimetric assay (B) and NMR titration spectra of molecular aptamer beacon (C).

Fig. 2 – Confocal Laser Scanning Microscopy (CLSM) images of PC-3 cell line with AS1411-N5 at incubation of 24h

Conclusions

• AS1411-N5 forms a parallel G4 conformation and did not show polymorphism, suggesting a single conformation, as shown in CD and NMR spectra
• High affinity of AS1411-N5 to NCL, as evidenced by Kd determination and co-localization evidenced in vitro assays;
• Higher NCL expression in PBMC cells of prostate cancer patients when compared to healthy volunteers;
• Higher NCL levels in advanced PCa patients suggesting that NCL quantification can be used as an alternative prognosis marker;
• AS1411-N5 recognizes and detect specifically NCL protein, even in complex samples;
• AS1411-N5 revealed to be a potential candidate to NCL detection in blood samples using a suitable microfluidic system.

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