SCIENCE*SPRING*DAL

Life Science Department

Development of a nanodiagnosis kit for polymorphisms associated with obesity

STABVIDA, Investigação e Serviços em Ciências Biológicas, Lda

NanoTheragnostics Group, CIGMH

Fábio Ferreira Carlos

Ph.D. Student since October 2010 (Supervisors: Pedro Viana Baptista/Gonçalo Doria/Orfeu Flroes)

2008 - 2010: Human Genetics Researcher in STABVIDA.
2002 - 2007: Graduated in Biology at ULHT.

Objectives

The aim is to develop a kit for characterization of polymorphisms associated with obesity using gold nanoprobes and evaluate its integration into a microfluidic platform for the genotyping in "Point-of-Care" (POC). There are four specific points:
i) Characterization of biological samples and selection of genes / SNPs relevant to the genetic predisposition to obesity, overweight and associated diseases (FTO rs9939609, PPARG rs1801282 and APOA5 rs662799);
ii) Synthesis of noble metal nanoprobes and characterization of polymorphisms selected by the method of non-cross-linking;
iii) Development and validation of the nanodiagnostics kit;
iv) Marketing Strategy.

Methodology

A total of 194 samples obesity related, will be characterized through the amplification (PCR) and direct Sequencing (Sanger) (Figure 1) to determine the relation between genotype vs phenotype (odd ratio values). Afterwards two sets of nanoprobes for the characterization of both alleles will be synthesized for detection of sequences via the non-cross-linking method. Stability studies will be performed in function of the system temperature, storage time, storage characteristics, conditioning reagents and conditions for the sample. The kit results will be compared with the current reference technique, direct sequencing, as regards sensitivity, specificity, accuracy, precision and detection limit. The microfluidic system will consist of three levels of processing: Isolation of the sample and its purification, amplification and hybridization of the target with nanoprobes, and revelation and reading results in colorimetric readout windows isolated.

Expected Results

Significant differences in allelic expression of FTO rs9939609, PPARG rs1801282 and APOA5 rs662799 between control and case groups, indicating if there is higher risk for obesity in presence of one or both risk alleles when comparing the control group with the entire obese group. The non-cross linking method will allow a visual comparison / spectrophotometric (Figure 2) the three test solutions (Positive, Negative and Blank) before and after the aggregation of the Au-nanoprobes After addition salt both, blank and negative acquire a blue color, thereby demonstrating aggregation of the Au-nanoprobes. While in the case of the positive control show no aggregation of the Au-nanoprobes maintaining the initial red color.
Integration of the nanodiagnostics system in a microfluidic prototype.
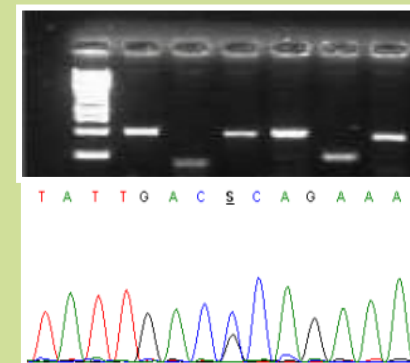
Figure 1 – Amplification and direct sequencing of obesity related samples.
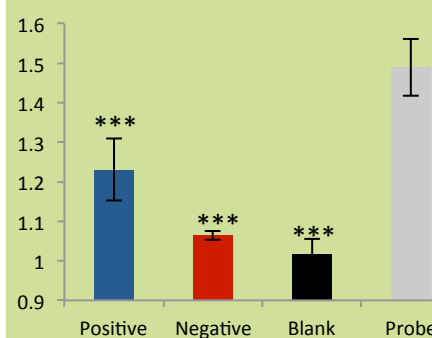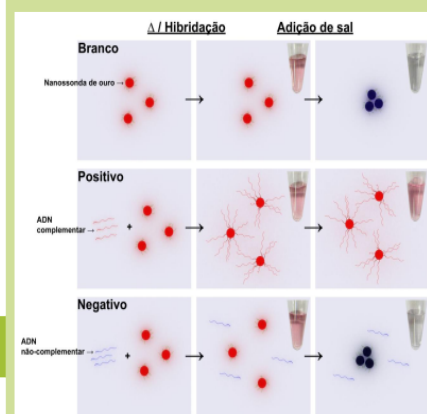
Figure 2 – Non-cross method linkking