SCIENCESPRINGDAY

FACULDADE DE CIÊNCIAS E TECNOLOGIA
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4 published papers
5 oral presentations
14 posters

Molecular Mechanisms underlying Hypertrophic Cardiomyopathy: from genes to miRs and proteins

Human Genetics Research Lab

Objectives

Main goal: To understand HCM genetic basis and simultaneously to identify new diagnostic biomarkers of HCM. In order to accomplish this we aim to:

i) Validate HRM as an effective technique for HCM genotyping by comparing this technology with targeted NGS;

ii) Establish *in vitro* functional assays for the pathological characterization of the novel identified mutations using cardiomyocyte cell lines.

iii) Characterize miR expression profiles in cardiac tissue to reveal novel miR-based pathways underlying the cardiac remodeling and identify their mRNA targets

iv) Design silencing assays by targeting deregulated miRs previously identified that are valuable for gene therapy using primary cardiomyocyte cell lines.

Methodology

- **Patients genotyping:** High Resolution Melting (Roche) *vs* Next Generation Sequencing (Life Technologies). (Figure 1)
  - 400 HCM-patients and nearly 200 genomic regions
- **Functional characterization of the novel HCM-causing mutations:** *in silico* and *in vitro* characterization: bio-informatics, mammalian two-hybrid assays, phenotype induction in cardiomyocytes cell culture. (Figure 2)
- **Characterization of miR expression profile in HCM:** profiling of 742 known miRs by Real-Time PCR and miR Deep Sequencing Analysis of Gene Expression profiling to reveal novel miRs.
- **miRs validation as HCM molecular biomarkers and potential therapeutic targets:** induction of aberrant miR expression in cardiomyocyte cell line and phenotype reversion.

Expected Results

- HCM patient genotyping show that in the Portuguese population the most mutated genes are *MYBPC3*, *MYH7* and *TNNT2*, all coding for sarcomeric proteins. (Figure 3)

- Functional studies for novel HCM-causing mutations in *MYBPC3* are on-going. We expect these mutations to interfere with normal sarcomeric functioning. (Figure 4)

- Preliminary miR expression studies showed 8 highly de-regulated (100x-1000x) miRs.

Funding: Private funding

Figure 1 – Patients Genotyping

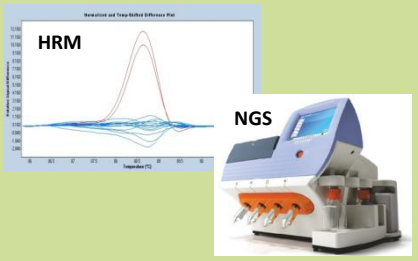
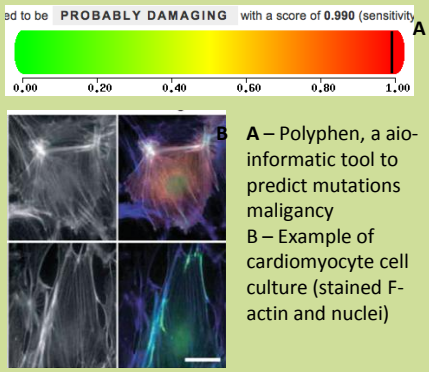


Figure 2 – Novel HCM-mutations characterization



A – Polyphen, a aio-informatic tool to predict mutations maligancy
B – Example of cardiomyocyte cell culture (stained F-actin and nuclei)

Figure 3 – Genotyping results in the Portuguese Population

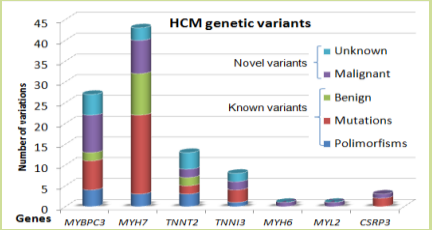


Figure 4 – Myosin binding protein C , encoded by *MYBPC3*, and interacting proteins. Mutated domains are highlighted in red.