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Introduction:

Mutations in sarcomere proteins are usually associated to cardiac diseases, including Hypertrophic Cardiomyopathy (HCM). In particular, the R92Q mutation in cardiac Troponin T gene (TNNT2) was found to be responsible for the development of HCM with a dominant hereditary trait.
The goal of my project was to set up an allele-specific silencing approach to inhibit the expression of the mutant R92Q allele, without suppressing the expression of the wild type TNNT2 protein. During the second year of the project, a number of siRNA targeting the R92Q mutation were designed and tested.

Methods:

A set of siRNA duplexes that carry the nucleotide variation G>A in the center of the sequence were designed and tested using the dual-luciferase reporter assay system. To this aim, partial sequences of the WT and mutant TNNT2 genes were inserted in the 3'-UTR regions of the *Photinus* and *Renilla luciferase* genes, cloned in the pGL3-TK and pRL-TK vectors, respectively. The designed siRNA, mutant and WT reporter alleles were co-transfected in cultured mammalian cells and the day after the dual luciferase assay was performed to evaluate the expression levels of luciferase.

Results:

We analyzed a total of seven siRNA targeting the R92Q mutation.
At first, three 21-mer siRNA duplex were tested; they induced an inhibition higher than 70% of both the WT and the mutated alleles. To increase the silencing efficiency and selectivity, we selected one of the three siRNA to be further modified. In particular, the siRNA was modified to a 19-mer siRNA duplex different mismatches in the seed region were introduced.
Following luciferase assay, among the novel siRNA designed, we identified a siRNA with a mismatch in position 13, able to induce a decrease of about 83,06 % the expression of the mutant allele and of only 11,5 % of the wild type allele.