OMICS Group Conferences
Accelerating Scientific Discovery

3rd International Conference on
Clinical & Experimental Cardiology

April 15-17, 2013 Hilton Chicago/Northbrook, USA

Oligonucleotide therapeutic approaches for allele silencing of hMYL2-47K and hMYH7-403Q mutations in hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a genetic disease of the heart muscle and the most common cause of sudden death in young people and athletes. It is caused by heterozygotic missense mutations in genes encoding proteins of the cardiac sarcomere. We hypothesized that the delivery of oligonucleotide reagents with allele-specific silencing capabilities might abrogate the negative effects of the disease. As proof of principle, we generated cell models by cloning wild type and mutated alleles fused to green and cherry fluorescent reporters into human embryonic kidney cells (HEK293). We targeted the following mutations: i) regulatory myosin light chain MYL2 (N47K), ii) beta myosin heavy chain MYH7 (R403Q, human), and iii) alpha myosin heavy chain MYH6 (R403Q, mouse). Using these models, we tested the efficacy and specificity of various siRNAs (non-viral delivery) and shRNAs (viral delivery) using fluorescence activated cell sorting (FACS) and quantitative RT-PCR to explore the dynamics of position specific mismatch. We have identified siRNAs & shRNAs with moderate efficacy and high specificity for human N47K and R403Q mutations. Human transgenic mouse model for N47K and knock-in mouse model for R403Q mutations are used to generate neonatal cardiomyocytes (CMs) for adeno-associated virus-9 transduction expressing shRNAs for *in vitro* and *in vivo* experimental studies, meanwhile for the human R403Q, we have generated induced pluripotent cells (iPSc) for cardiomyocyte differentiation. To assess the contractility changes produced after treatment with shRNAs, the mechanical properties of cardiomyocytes are measured by using a new polyhydrogel imaged-based functional assays (IFAs) that detects changes in CMs mechanical function such as contraction and relaxation velocity. We expect that the silencing of the mutant-allele will allow the expression of the allele-wild type gene reverting the functionality of the cells. The outcomes of these studies will provide useful information for the development of novel therapeutics for cardiovascular diseases.

Biography

Kathia Zaleta completed his Ph.D. from the National Polytechnic Institute in Mexico City in collaboration with the Chemistry Department at Nebraska University. She completed her postdoctoral fellowship in Chemistry from Stanford. During her Ph.D. and postdoctoral career she was investigating the catalytic mechanisms of modular megasynthases such as polyketide synthases (PKS) and the Non-ribosomal peptide synthetases (NRPS), with the concomitant goal of harnessing their programmable chemistry for preparing novel pharmaceutically relevant natural products. This work allowed the bases for the development and design of custom drugs by genetic manipulations of the original gene cluster to create a new biosynthetic mechanism and so on a novel molecule drug with biological activity. Her current research involves designing new gene-based therapeutic drugs to cure the inherited heart disease Hypertrophic Cardiomyopathy (HCM).

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