Consiglio Nazionale delle Ricerche

Istituto di Biologia Cellulare e Neurobiologia

Institute of Cell Biology and Neurobiology

CNR-IBCN

AVVISO DI SEMINARIO

SEMINAR ANNOUNCEMENT

Improving AAV-mediated, CRISPR/Cas9-induced homologous recombination

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International Centre for Genetic Engineering and Biotechnology

Padriciano –Trieste

01 December 2017, 14:00 – 15:30

Monterotondo CNR Seminar Room, Building 21

Highlights: The definitive treatment of genetic diseases through precise gene editing has been a long sought goal of gene therapy, unachieved at clinical level yet. The advent of biotechnological tools that use the bacterial CRISPR-Cas9 endonuclease and an engineered single-guide RNA (sgRNA) for the manipulation of mammalian genomes has dramatically changed this perspectives. Despite the overall excitement, however, gene correction through the precise homologous recombination (HR) machinery is largely less efficient than the error prone non-homologous end joining (NHEJ) in mammals, in particular in adult post-mitotic tissues, such as heart and brain. For this reason, we designed an in vitro fluorescence-based assay for the genome-wide, high-throughput identification of RNA regulators and enhancers of gene editing mediated by HR in U2OS cells. We identified 21 microRNAs mimics that significantly increased HR events compared to controls ($P<0.001$). Additionally, a positive effect on HR efficiency was also observed in cultures of primary neonatal rat cardiomyocytes, using the same reporter constructs adapted to an AAV6 vector system. While we are now investigating on the molecular mechanisms leading to enhance efficiency of HR, these findings encourage us in pursuing microRNA delivery as a tool to achieve gene correction at clinically relevant levels. To this purpose, we have design gene editing strategies to correct an autosomal dominant genetic defect in a mouse model of a human hypertrophic cardiomyopathy (HCM), caused by a point mutation in the sarcomeric MYBPC3 protein. Promising preliminary results confirm the potency of CRISPR-Cas9 endonuclease combined with the efficiency of AAV vectors for cardiac gene transfer.

Host: Prof. Fabio Mammano

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