TACKLE ATHEROSCLEROSIS VIA GENOME EDITING: CRISPR MEETS ApoA-I MILANO

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Atherosclerosis is one of the most important health issues in western world. It is determined by bloodstream lipids excess, and their deposition in to the so-called atherosclerotic plaque, which in turn is responsible for partial or total artery occlusion, affecting mainly carotid and coronary vessels. Unlike the larger lipoprotein particles which deliver fat molecules to cells, high-density lipoproteins (HDL) particles remove lipids from cells. Apolipoprotein A1 (ApoA-I), the major protein component of HDL particles in plasma, enables efflux of lipids from cells to transport elsewhere, including back to LDL particles or to the liver for excretion. ApoA-I Milano is a naturally occurring mutated variant of the ApoA-I protein found in human HDL. ApoA-I Milano has been shown to reduce atherosclerosis in animal models and in a small phase 2 human trial. Recombinant adeno-associated virus 8 (AAV8) mediated ApoA-I Milano gene therapy in combination with low-cholesterol diet induces rapid and significant regression of atherosclerosis in mice. Due to its enormous apparent efficacy, some have speculated that development of synthetic ApoA-I Milano may be a key factor in eradicating coronary heart disease. Despite this, when comes to produce a synthetic ApoA-I Milano protein, several limitations occur, being the process technically challenging, time consuming, and expensive. On the other hand, AAV8-based gene transfer approach is affected mainly by the need of repeated cycles of treatment.

CRISPR/Cas system (a prokaryotic adaptive immune response system) uses non-coding RNAs (sgRNA) to guide the Cas9 nuclease to induce site-specific DNA cleavage (double strand break, DSB). These damages will be repaired by cellular DNA repair mechanisms, via non-homologous end joining or homology directed repair (HDR) if a donor DNA is provided to the cells.

In light of what afore mentioned, a CRISPR-based strategy was designed to overcome the limitations of both synthetic ApoA-I Milano, and gene transfer, in order to assess if we could let wild type hepatocytes to acquire the ApoA-I Milano genotype and produce a mutated ApoA-I protein.

We performed a first batch of in vitro experiments, on HepG2 human hepatocytes and on primary hepatocytes from C57BL/6-Tg(APOA1)1Rub/J (HepRub/J) transgenic mice, carrying the human ApoA-I gene instead of the murine homologue. First, we designed a sgRNA targeting the ApoA-I gene (to let Cas9 produce a DSB) and a donor DNA carrying the Milano mutation (DNA-Milano) flanked by two regions of homology (which will be used by the cells to repair the Cas9-induced DSB). Second, we cloned the ApoA-I targeting sgRNA into a Cas9-expressing plasmid (px330 from Addgene including the ApoA-I sgRNA, hereinafter px330ApoA-I); third, we transfected both HepG2 and HepRub/J cells with the px330ApoA-I vector alone or in combination with DNA-Milano. After negative selection, we screened our cells via direct sequencing, to check for the Milano mutation.

To estimate the relative amount of ApoA-I Milano produced by our cells, we performed both RT-qPCR and direct quantification.

Our results indicate that by using CRISPR we can efficiently target ApoA-I human gene and edit its sequence in order to obtain a Milano genotype, at least in vitro.

We are currently engaged in in vivo experiments on C57BL/6-Tg(APOA1)1Rub/J mice, to verify if we could achieve the same results by delivering the genome editing system via AAV vectors targeting liver tissue.