Enabling Cholesterol Catabolism in Human Cells

Awardee: Richard E. Honkanen
Award: Transformative Research Award
Awardee Institution: University of South Alabama
Co-authors: Mark R. Swingle, Alla Musiyenko, Brandon D'Arcy, Kevin Abney
Co-authors institution: University of South Alabama

Cardiovascular disease (CVD) is the leading cause of death in the United States, and the cost associated with the medical management of CVD is staggering (>$444 billion in 2010[a]). CVD is complex and can originate from many different aberrations in normal lipid metabolism (some genetic, some lifestyle choices). However, nearly all forms of CVD are associated with elevated plasma lipoproteins (principally LDLs) often in combination with low levels of high-density lipoproteins (HDLs). For many people, CVD is an age dependent, progressive disease that is largely undetected or ignored until an event (i.e. myocardial infarction) occurs in the later stages of disease. Therefore, current therapies focus on preventing a second event (or a primary event in high risk individuals) by reducing the circulating levels of LDLs and increasing levels of HDLs. At a biochemical level, the inability of human cells to degrade the cholestane ring of cholesterol is a fundamental component of CVD. More precisely, if macrophages had the ability to degrade cholesterol, they would not become engorged with cholesterol/cholesterol esters and elicit the maladaptive immune response that leads to the onset and progression of atherosclerosis. The surprising observation that chronic *Mycobacteria* survival in human macrophages was aided by their ability to use phagosome cholesterol as a carbon and energy source lead us to a novel hypothesis: genes encoding bacterial ring opening enzymes can be humanized and used to transformation human monocyte derived macrophages, enabling the degradation of phagosome-cholesterol. To test this hypothesis, we have humanized several key enzymes, including cholesterol dehydrogenase, 3-ketosteroid-Δ1-dehydrogenase (KSTD), 3-ketosteroid-9-αhydroxylase (KshA/B), and cholest-4-en-3-one-Δ^1-dehydrogenase. Together these enzymes can catalyze B-ring opening and aromatization of ring A. To control the expression of the enzymes in our "cholesterol catabolizing gene cassette," we are developing two expression systems for comparison. One is a transcription activator-like effector nuclease (TALENs) targeting the AAVS1 locus in the human genome. The second is a Cas-9 nuclease based system designed to introduce the cassette into the ABCA1 promoter (a lipid flipase that is induced by elevated cholesterol). To coordinate the expression of this multi-enzyme system, we have adapted a "ribosomal skipping" sequence between proteins. This eliminates the need for multiple promoters to control the expression of each protein, which greatly reduces the size of the expression cassette.

[a]http://www.cdc.gov/chronicdisease/resources/publications/AAG/dhdsp.htm

NIH COMMON FUND HIGH-RISK HIGH-REWARD RESEARCH SYMPOSIUM
DECEMBER 15 – 17, 2014
POSTER ABSTRACTS – SESSION 2 (DEC. 16, 2014)