

## **AAV GENE THERAPY FOR ALCOHOLISM: INHIBITION OF MITOCHONDRIAL ALDEHYDE DEHYDROGENASE ENZYME EXPRESSION IN HEPATOMA CELLS.**

Anamaria C. Sanchez, Centre for Biotechnology and Bioengineering (CeBiB), Department of Chemical Engineering and Biotechnology, University of Chile, Chile.

anitaasd@gmail.com

Chengwen Li, Gene Therapy Center, University of North Carolina, Chapel Hill, NC 27599 R.

Jude Samulski, Gene Therapy Center, University of North Carolina, Chapel Hill, NC 27599

Barbara Andrews, Centre for Biotechnology and Bioengineering (CeBiB), Department of Chemical Engineering and Biotechnology, University of Chile, Chile.

Juan A. Asenjo, Centre for Biotechnology and Bioengineering (CeBiB), Department of Chemical Engineering and Biotechnology, University of Chile, Chile.

**Key Words:** Gene Therapy, AAV, Alcoholism, ALDH2, shRNA

The process by which ethanol is metabolized in the liver occurs in two steps. The first step depends on the enzyme alcohol dehydrogenase (ADH) and the second step is catalyzed by the enzyme Aldehyde dehydrogenase (ALDH2). Some individuals of the Asian population who carry a mutation in the Aldehyde dehydrogenase gene (ALDH2\*2) have a diminished capacity to metabolize acetaldehyde, producing strong effects including facial flushing, dizziness, hypotension, and palpitations. This results in an aversion to alcohol intake and protection against alcoholism. The large prevalence of this mutation in the human population strongly suggests that modulation of ALDH2\*2 by genetic technologies could result in a similar phenotype. We utilized scAAV2 vectors encoding ALDH2 shRNA to validate this hypothesis by silencing ALDH2 gene expression in human cell lines. In the present study, we have shown that scAAV2 vectors encoding a single ALDH2 shRNA are effective in decreasing mitochondrial Aldehyde dehydrogenase expression in HEK-293/ALDH2, HepG2 and VL-17A HepG2 cell lines and increases acetaldehyde levels. Human cell lines 293 and HepG2 were transduced with scAAV2/shRNA showing a reduction in ALDH2 RNA and protein expression with the two viral concentrations assayed (1x10<sup>4</sup> and 1x10<sup>5</sup> vg/cell) at two different time points. In both cell lines ALDH2 RNA levels were reduced by 90% and protein expression was inhibited by 48% and 90%, respectively, five days post infection. ALDH2 silencing was evaluated in the VL-17A HepG2 cell line, which exhibits hepatocyte-like characteristics in response to ethanol. Cells were transduced with 1x10<sup>5</sup> vg/cell and ALDH2 expression was evaluated at the third day p.i. by Real Time RT-PCR and Western blot, showing an expression reduction of 40% as compared with the scramble control, in both analysis. As functional assay cells were incubated with ethanol (10, 25 and 100 mM) and acetaldehyde accumulation was measured by gas chromatography. Samples treated with the scAAV2/shRNA virus showed an increase of acetaldehyde levels of 50, 30 and 40% for each ethanol concentration assayed. Previously we have demonstrated a 50% decrease in ethanol consumption over 35 days with a similar gene therapy treatment of alcoholic mice that also inhibited the AIDH2 gene. These results suggest that gene therapy could be a useful tool for the treatment of alcoholism by knocking down ALDH2 expression using shRNA technology delivered by AAV vectors.