THE IMPORTANCE OF microRNA BIOLOGY TO YOUR RESEARCH: WHAT, WHY AND HOW

Orna Issler, Alon Chen

Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

miRNAs are a subset of endogenous small RNA molecules that regulate gene expression post-transcriptionally by affecting mRNA stability or translational repression. The study of this recently discovered family of genes is an exponentially growing field in life sciences and medical research. Diverse methods have been developed for the study of miRNA involvement both under normal and pathological conditions. Bioinformatics tools enables prediction of miR- target interaction for studying the regulation of any gene of interest based on conservation data, and these predictions can be verified by *In vitro* assays. High throughput methods for the profiling of miRNA expression patterns as microarrays or deep sequencing are available, suggesting potential miRNAs as biomarkers for specific physiological conditions. In the whole animal context, modified protocols for *in Situ* hybridization and real time PCR are often used to explore miR expression pattern in specific tissues. Transgenic mouse models for miRNA reporter, knockout or over-expression are generated as mouse models for miRNA-based diseases. Finally human studies include SNP analysis and the measurement of circulating miRNAs and their correlation with pathological conditions. Implementation of miRNA technologies in research opens new possibilities for thrilling discoveries.

INTRODUCTION TO OPTOGENETICS: THE FUTURE IS ALREADY HERE

Sharon Haramati, Alon Chen

Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

Halorhodopsin (NpHR) and Channelrhodopsin-2 (ChR2) are recombinant light-gated ion channels that transport chloride ions or cations, respectively. Each channel is selectively activated by different wavelength of light. These channels can be used for the precise control over neuronal activation or inhibition in genetically altered neuronal cells. These (and similar) channels have been studied intensively in recent years in various model systems such as: neuronal cell cultures, brain slices, in-vivo in transgenic animals such as c.elegans and mice and in mammals that were virally infected to express these opsins.

The electrophysiological properties of these transporters are well elucidated and they were found to be non-toxic, wavelength specific and very precisely controlled by the light stimulus as set by the experimenter (up to single-spike resolution).

This genetic based technique offers an exciting opportunity for the study of the neural substrates of behavior. By genetically manipulating certain cell types or brain structures to express these channels and together with an optic setup enabling the delivery of wave-length specific light onto desired brain locations, one can switch on or off, reversibly and in a very precisely controlled manner, certain neural circuits in the mammalian brain while it is engaged in a task.

GUTLESS ADENO ASSOCIATED VIRUS FAMILY: A NEW TOOL AND POTENTIAL TREATMENT PARADIGM FOR THREE ENDOCRINE SYSTEMS

Gary Weisinger[1], Meital Grafi-Cohen[1], Judith Rein-Rondel[1], Ariel Many[2], Rona Limor[1], Zaki Kraiem[3] and Naftali Stern[1]

[1]Departments of Endocrinology, Metabolism and Hypertension as well as [2]Obstetrics, Tel Aviv Sourasky Medical Center and the Sackler Faculty of Medicine; [3]Faculty of Medicine, Technion, Haifa

Background: Traditionally genetic manipulation *invitro* has involved the transfection, electroporation or lipofection of plasmids into cell lines appropriate for the particular question. Unfortunately the ability to use these techniques in primary cultures and *in vivo* is usually not transferable. To deal with this issue different viral based systems have been developed that are able to infect primary systems *invitro* or *in vivo*. The search for the optimal viral vector system has been the challenge for many investigators. We present here an introduction to the Gutless recombinant Adeno-Associated Virus (rAAV) as we have applied it to some endocrine related projects. Gutless rAAV vectors are considered safe for *in vivo* use for both animals and humans. Over the last decade more than 17 human clinical have been initiated using different rAAV serotypes. Each serotype has a different range of tissue specificities.

Aim: In the current presentation our aim was to screen 5 different rAAV serotypes in primary adipose, smooth muscle and thyroid tissue in culture, in order to determine which would be the most efficient and effective for further genetic manipulation. Furthermore, we wish to demonstrate an application of the most optimal rAAV serotype in cultured vascular smooth muscle cells (VSMC).

Methods: Primary human preadipocytes (HPA), VSMC and primary human thyroid cells were prepared from tissue taken during operations under local Helsinki supervision. Cells were transduced with 5 rAAV serotypes all with the ability to express nuclear directed eGFP upon cell infection. Infection efficiency was determined by the % of green fluorescent cells counted at different time periods. Furthermore, VSMC were infected with the optimal rAAV serotype able to express a 12-lipoxygenase (12-Lo) knockdown sequence under the CMV promoter. In this case VSMC cell death was measured by trypan blue exclusion and visual estimation.

Results: HPA were optimally infected by AAVDJ, which showed strong maximal eGFP expression from 6-30 days post infection.
VSMC were optimally infected by both AAV2 and AAVDJ, with maximal eGFP expression over 6-10 days and 10-70 days respectively.
Human primary thyroid cells in culture were optimally infected by AAV12 with a maximal eGFP expression 3 days after infection.
12-Lo knockdown AAV2 particles induced massive cell death with a similar time course as eGFP expression. This VSMC death was preventable by the addition of the 12-Lo product, 12HETE with the virus. Control vectors did not kill VSMC.

Conclusions: Once optimized, these vectors may prove useful new tools for the future treatment of human vascular disease. The gutless rAAV family of viruses will be useful tools for future endocrine research.