European Society of Gene and Cell Therapy
French Society of Cell and Gene Therapy
Collaborative Congress 2012

October 25–29, 2012
Palais des Congrès de Versailles
Versailles, France

hCMV > CMV > hCMV/RPE65 in RPE cells, detectable up to 3 months of culture. In vivo GFP expression was consistent with the in vitro results and observable for at least 32 dpi.

pEPito-based vectors containing S/MARs and reduced CpG content are active expression systems in RPE cells for up to 100 cell divisions. In vivo, gene expression is detectable for at least 32 days. Based on its efficiency, the vector with hCMV promoter has been selected for further administration of a therapeutic molecule in a mouse model of retinal neovascularization.

Support: IBB/LA; PEst-OE/EQB/LA0023/2011; PIRG-GA-2009-249314; FCT Portugal (SFRH/BD/76873/2011,SFRH/BD/70318/2010).

P236

An homologous model of gene therapy by *in vivo* administration of a plasmid containing the mouse growth hormone gene in immunocompetent dwarf mice

CR Cecchi[1,2], F Dagnaes-Hansen[2], L Aagaard[2], M Jakobsen[2], H Gissel[2], A Gothelf[3], BH Hansen[2], J Frystyk[4], E Higuti[1], NAJ Oliveira[1], ER Lima[1], P Bartolini[1], TG Jensen[2], CN Peroni[1]

[1]Biotechnology Center, IPEN-CNEN, University of São Paulo, São Paulo, SP, Brazil, [2]Department of Biomedicine, Aarhus University, Aarhus, Denmark, [3]Center for Experimental Drug and Gene Electrotransfer, Department of Oncology, Copenhagen University Hospital Herlev, Herlev, Denmark, [4]The Medical Research Laboratories, Clinical Institute and Medical Department M (Diabetes and Endocrinology), Aarhus University Hospital, Aarhus, Denmark

An alternative method for growth hormone deficiency (GHD) treatment has been developed using gene therapy associated to electroporation. Sustained circulatory levels of hGH and a highly significant weight increase were obtained in immunodeficient dwarf (lit/scid) mice. This new study aimed at setting up an homologous model of gene therapy based on electrotransfer of genomic mouse growth hormone (gmGH) in the muscle of immunocompetent dwarf (lit/lit) mice. The plasmid pUC-UBI-gmGH was first transfected into HEK 293 cells and an *in vitro* expression up to 74.0 ng mGH/10^6 cells/day, compared to 6.0 ng mGH/10^6 cells/day for the negative control, was obtained. As a positive control for *in vivo* expression, this plasmid was administered as naked DNA via hydrodynamic injection into C57BL6 normal mice, and the weight increase of treated mice after 21 days was 60.10% ± 11.02% compared to −2.05% ± 2.32% for the control group (injected with Ringer's solution). The plasmid was then administered in a protocol of gene-electrotransfer, applying eight 150 V/cm pulses of 20 ms, for a body weight gain assay into lit/lit mice. The weight increase after 15 days was 13.48% ± 3.53% for the gmGH-treated group versus 5.66% ± 2.51% for the control group (injected with saline) and 13.10% ± 7.54% for the group injected with the same plasmid encoding the ghGH gene, both groups also followed by electroporation. This previous results can be considered very promising as a pre-clinical study in a gene therapy protocol for patients suffering for GHD.

Supported by: CNPq and FAPESP.

P237

Effects of antibiotic pretreatment and bacteria-mediated reprogramming on inflammatory bowel disease in mice.

P Celec, R Gardlík

Institute of Molecular Biomedicine, Comenius University, Bratislava, Slovakia

Since the original study of Takahashi and Yamanaka in 2006 the field of induced pluripotent stemcells has made a great progress. However, all of the experiments published so far are based on ex vivo gene delivery and subsequent reimplantation of the cells. Compared to that, in vivo reprogramming might benefit from the direct administration of DNA encoding the reprogramming factors into the target tissue. In our previous experiment we proved some beneficial effects of bacteria-mediated delivery of genes encoding reprogramming factors Sox2, Oct3/4 and Klf4 on the course of colitis in mice. Preventive oral administration of the modified strain Salmonella Typhimurium SL7207 resulted in improvements in weight loss, colon length and stool consistency. Recently it has been shown that antibiotic pretreatment can alleviate the course of chemically induced colitis in mice. In the current study, we tested whether the antibiotic pretreatment of mice could result in better colonization of administered bacterial strain of colon, more effective gene delivery, cell reprogramming and, thus, also stronger therapeutic outcome. Mice C57BL/6was given streptomycin and metronidazole for 4 days before multiple oral administrations of therapeutic bacteria every other day. After two applications, mice were given dextran sulfate sodium in drinking water to induce colitis. Disease activity parameters, such as stool consistency, weight loss and bleeding were monitored throughout the experiment. Our results indicate that antibiotic pretreatment might alter the bacterial gene delivery into the colon. Moreover, in vivo reprogramming of colon cells seems to have an effect on the course of colitis.

P238

Reduced in vitro and in vivo toxicity of siRNA-lipoplexes with addition of Polyglutamate

Virginie Escriou[1], Anne Schlegel[1,2], Pascal Bigey[1], Daniel Scherman[0]

[1]UPCGI CNRS 8151 Inserm 1022 ParisDescartes ENSCP Chimie ParisTech, Paris, France, [2]DNA Therapeutics, Evry, France

We previously designed a new siRNA vector that efficiently silences genes in vitro and in vivo. The vector originality is based on the fact that, in addition to the siRNA molecule, it contains two components: 1) a cationic liposome which auto-associates to the siRNA to form particles called "lipoplexes", 2) an anionic polymer which enhances the lipoplex's efficiency. This anionic polymer can be a nucleic acid, a polypeptide or a polysaccharide. We show here how the nature of the added anionic polymer into our siRNA delivery system impact the toxic effects induced by siRNA lipoplexes. We first observed that: (i) siRNA lipoplexes-induced toxicity was cell line dependent, tumoral cell lines being the more sensitive, (ii) pDNA-containing lipoplexes were more toxic than polyglutamate-containing ones or cationic liposomes. We next determined that the toxicity induced by plasmid-containing lipoplexes is a long-lasting effect that decreased survival capacity of cells for several generations. We also found that treated cells underwent death following apoptosis pathway. We then examined the siRNA lipoplexes induced toxicity following systemic injection to mice and observed that (i) injection of siRNA-lipoplexes, rather than of liposome, triggered a production of several cytokines in mice and (ii) replacement of plasmid by polyglutamate reduced the elevation of all the assayed cytokines.