understanding the role of cilia in other forms of ciliopathy. Further optimization will be needed to increase efficiency of transduction, document stability of transgene expression and evaluate potential toxicities.

144. Sustained Hgh Expression after Electrotransfer of Naked DNA into Dwarf "Little" Mouse Skeletal Muscle

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Ex vivo growth hormone gene therapy based on retrovirally transduced primary human keratinocytes has led, in our previous work, to relatively high circulating levels of human (hGH) or mouse growth hormone (mGH) in "little" mice (lit/lit) . Unfortunately these levels fell to baseline within a short period of time. The use of non-viral vectors is an alternative to ex vivo Gene Therapy based on viral vectors. Non-viral vectors are safer and less expensive, but also less effective than viral vectors. Electroporation, or electrotransfer, is a strategy used to increase the delivery of plasmid DNA intramuscularly or intradermically. This study aimed to verify the feasibility of the electrotransfer technique for increasing the efficiency of DNA intramuscular injection , using a plasmid containing the hGH gene. The pUC-UBI-hGH plasmid used in this study contains the ubiquitin C promoter and the genomic hGH sequence. "Little" mice were anesthetized followed by a hyaluronidase injection (20 U / 50µl) in the quadriceps muscle region. After 30 minutes, different amounts (12. 5; 25; 50; 75; 100µg) of purified plasmid DNA were administered, followed by electrotransfer using eight 50V pulses of 20 ms and 0.5 s of interval.

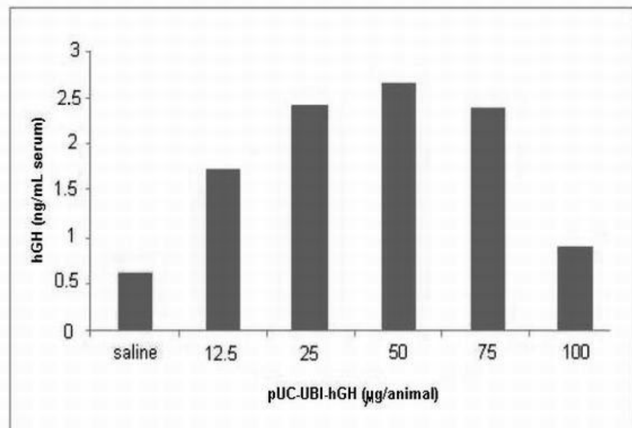

Figure 1: hGH levels in sera from lit/lit mice after three days (n = 3 animals per dose) from the injection of different amounts of plasmid DNA, followed by electrotransfer using eight 50V pulses of 20 ms with 0.5 s of interval. The dose-response curve, from 0 to 50 µg DNA, was: Y=0.036X+1.11 r=0.806 P<0.01 (n=11).

Blood was collected from the retro-orbital cavity after three days and hGH levels in the sera were determined by radioimmunoassay. We can observe that the dose-response curve presented a highly significant correlation (P<0.01) in the 0-50 µg range. In a second experiment, 50 µg of purified plasmid were administered as described, blood being collected after 1, 3, 6, 9 and 12 days. According to preliminary data, circulating levels of 2-3 ng hGH/mL were maintained for at least 12 days in the immunocompetent "little" mouse.

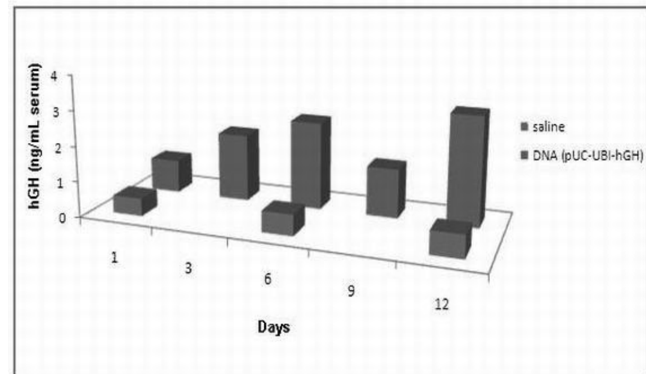

Figure 2: Expression of hGH over a 12 days period after administration of 50µg/animal of pUC-UBI-hGH plasmid followed by electrotransfer, using eight 50V pulses of 20 ms with 0.5 s of interval, in little mice (n=3 animals/condition). Each animal was used once and then sacrificed.

Such hGH levels have never been reported for naked DNA muscle injection. We intend now to verify the maximum period of permanence and in vivo bioactivity. In conclusion, we can say that the use of this methodology is quite promising for the development of new models of in vivo gene therapy for growth hormone.

145. Gene Transfer to the Cerebellum Using SV40-Derived Vectors

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Gene transfer to CNS has been studied using various vectors. Several conditions involving the cerebellum could be candidate for gene transfer/therapy. In these studies, we tested the effectiveness of gene expression delivered by vector administration in the cerebellum. Recombinant Tag-deleted SV40-derived vectors (rSV40s) transduce neurons and microglia very effectively in vitro, and in vivo following direct stereotaxic inoculation into the basal ganglia or cortex. We therefore tested rSV40 gene transfer to the cerebellum in vivo. We characterized the distribution, duration and cell types transduced. For these studies, we used a rSV40 vector carrying HIV-Nef with a C-terminal FLAG epitope. An unrelated rSV40 vector, SV(BUGT), was used as a rSV40 control. Sprague-Dawley rats were given control and test vectors stereotaxically into the cerebellum. Transgene expression was studied 1, 2 and 4 weeks after cerebellum injection by immunostaining of serial brain sections. After administration of the vector, FLAG epitope-expressing cells were seen, at all time points after vector administration. Transgene expression was principally detected in the layer of the Purkinje cells of the cerebellum. No FLAG epitope-positive cells were seen when SV(BUGT) was injected. Coimmunostaining for lineage markers showed that neurons were the main cells transduced. These were mostly Purkinje cells, identified as immunopositive for calbindin. More rarely, microglial cells were tranduced; transgene expression was not detected in astrocytes and oligodendrocytes. These data demonstrate that administration of SV40-derived vectors can provide effective transgene delivery and expression in the cerebellum.

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