and mutant huntingtin alleles will be tolerated in humans, we investigated an allele-specific RNAi approach for HD. Here we show two different strategies for specific silencing of the mutant huntingtin allele. One is based on structural changes of the mutant huntingtin mRNA as a consequence of the massive CAG-expansion within exon 1 relative to the wild type allele. A second approach is based on a polymorphism in exon 58 that reportedly resides in ~40% of mutant huntingtin alleles in a sample South American population. To perform our analyses, we developed a novel assay design. Briefly, we generated two plasmids expressing full-length wild type or mutant huntingtin (CAG expansion in exon 1 plus exon 58 polymorphism), each possessing distinct epitope tags and either renilla (WT htt) or firefly (mutant htt) luciferase to normalize transfection efficiencies. This design allowed us to assess allele specificity in the same cell after co-transfection. Sequences of short interfering RNA (siRNA) targeting 5' and 3' of the CAG-repeat region, or the exon 58 polymorphism, were generated; some siRNA sequences were altered according to recent guide-strand rules for optimized RISC loading. Our initial studies with sequences targeting either the exon-58 polymorphism or the CAG repeat region showed specific silencing of the mutant allele. With some siRNAs, mutant protein levels were reduced to 60% with no reduction in wildtype protein levels. Other siRNAs preferentially silenced the mutant huntingtin allele up to 70% while reducing wild type protein levels to only 30% of control values. Together, our data indicate that targeting sequences flanking the CAG-repeat expansion region in exon-1, or the polymorphism in exon 58, may be viable strategies for allele specific silencing for HD therapy.

712. AAV-Mediated Allele-Specific RNA Interference of a Common Dominant Rhodopsin Mutation Causing Retinitis Pigmentosa

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Inherited retinal degenerations are a group of clinically and genetically heterogeneous diseases characterized by progressive photoreceptor cell death eventually leading to blindness and for which no therapies are available. Mutations in the rhodopsin gene are common causes of autosomal dominant retinitis pigmentosa (RP). Among them the P23H amino-acid substitution represents the most frequent rhodopsin mutation in US. Given the gain of function effect exerted by the P23H mutation, strategies aimed at silencing the expression of the mutated allele, like RNA interference, are desirable.

We have designed five different small interfering RNAs (siRNA) to specifically silence the P23H rhodopsin allele expressed by a transgene rat model of the disease. Following transient transfection in 293 cells, we have selected one siRNA with strongest rhodopsin P23H silencing ability. To allow continuous siRNA production bypassing the limit of repeated administrations otherwise required to obtain long term therapeutic effects, we have developed an adeno-associated viral (AAV) vector expressing the short hairpin RNA (shRNA) based on the siRNA selected *in vitro*. We show *in vitro* the ability of the AAV-expressed shRNA to efficiently inhibit the expression of the P23H but not wild-type rhodopsin allele at both protein and RNA levels.

Following administration of the AAV5 vector to the subretinal space of P23H transgenic rats, we show expression of the shRNA in the retina resulting in inhibition of rhodopsin P23H expression that is not able to prevent or inhibit photoreceptor degeneration. Since rhodopsin is the most abundant rod photoreceptor protein, systems resulting in more robust shRNA expression in the retina than the U1 small-nuclear RNA promoter or AAV serotype we used might be required to achieve therapeutic efficacy *in vivo*.

713. NFkB Decoy Oligodeoxynucleotides Inhibit Osteoclast Differentiation and Activation In Vitro and Improve Osteoporosis of Ovariectomized and Vitamin C Difficient Rat Models

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In this study, we focused on NFkB decoy oligodeoxynucleotides (NFkB decoy) as a new therapeutic strategy to attenuate osteoporosis. Tartrate resistant acid phosphatase (TRAP) positive multinucleated cells (osteoclasts) formed from neonatal rabbit bone marrow culture by 1,25-vitamin D3 stimulation were decreased by NFkB decoy treatment in dose dependent manner and pits formed on dentine slices by mature osteoclasts were reduced. In M-CSF and RANKL stimulated osteoclast culture system, NFkB decoy also inhibited the diffentiation and activation of osteoclast as well. In rat ovariectomized model of estrogen deficiency, continuous administration of NFkB decoy using osmotic pump attenuated the increase of TRAP activity, accompanied by a singificant increase in calcium concentration in femur and tibia and decreased in urinary deoxypyridinoline. In additional osteoporosis model of vitamin C deficient rat, inhibiton of NFkB by decoy dramatically improved the bone length, weight and mineral density as assessed by dual-energy X-ray absorptiometry. Overall, inhibition of NFkB by decoy strategy prevented osteoporosis through the inhibition of bone resorption. Targetting of NFkB might be potential therapy in various bone metabolic diseases.

714. Effect of Adenovirus-Mediated RNA Interference on Endogenous micro-RNA Functionality in a Mouse Model of Multidrug Resistance Protein-2 Gene Silencing

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RNA interference using viral vectors that express short hairpin RNAs (shRNAs) has emerged as a powerful tool for functional genomics and therapeutic purposes. However, little is known about shRNA in vivo processing, accumulation, functional kinetics and side-effects related to shRNA saturation of the cellular gene silencing machinery. Therefore, we constructed first generation recombinant adenoviruses encoding different shRNAs against murine Multidrug-Resistance Protein 2 (MRP2), which is involved in liver transport of bilirubin to bile, and analyzed MRP2 silencing kinetics. C57/BL6 mice injected with these viruses showed significant impairment of MRP2 function for up to three weeks, as reflected by increased serum bilirubin levels. The lack of MRP2 function correlated with a specific reduction of MRP2 mRNA and with high levels of processed shRNAs targeting MRP2. Inhibition was lost at longer times post-infection, correlating with a decrease in the accumulation of processed shRNAs. This finding suggests that there is minimal amount of processed shRNAs required for efficient silencing in vivo. This