Noxa and TRAIL and more cancer cell death than whole-genome RNA (approximately 15 kb) of complete Cantell strain without DI particles. Furthermore, we examined whether a specific structure of the DI RNA genome stimulates the RIG-I/MAVS downstream-related cancer suppressive pathways using HVJ-derived *in vitro* transcribed (IVT) RNAs. IVT-B2 which is derived from Cantell HVJ DI genome has a special secondary structure with a double-stranded RNA terminus and a single-stranded RNA loop. This IVT-B2 strongly stimulated RIG-I dependent proapoptotic proteins induction in prostate cancer cells. Modified IVT-B2 RNAs which had shorter dsRNA stem lost cancer cell killing activity and proapoptotic gene expressions. On the other hand, other modified IVT-RNA which had deleted ssRNA region in loop structure did not induce cancer cell-selective killing. We also found that calf intestinal alkaline-phosphatase-treated IVT-B2 RNA lost capability of inducing RIG-I/MAVS-related downstream Noxa and TRAIL expression. Finally, *in vivo* electroporation of IVT-B2 RNA induced intra-tumoral apoptosis and tumor suppression in human prostate cancer cell-xenograft mouse model by both direct tumor-cell killing and NK cell activation. These findings provide a novel nucleic acid medicine for the cancer treatment.

150. Further Development of an Allele-Specific Gene Silencing Strategy to Correct a Dominant-Negative Mutation Causing Collagen VI-Related Muscular Dystrophy

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Collagen VI-related congenital muscular dystrophies (COL6-RD), caused by mutations in any of the three genes coding for collagen type VI (*COL6A1*, *COL6A2*, *COL6A3*), underlie a spectrum of disorders ranging from severe life-threatening early onset Ullrich muscular dystrophy via intermediate phenotypes to the milder Bethlem type, typically presenting with generalized muscle weakness, proximal joint contractures, and respiratory failure. At present, there are no treatment options available for individuals affected with these diseases. The fact that the majority of COL6-RD cases are carriers of inherited or *de novo* dominant mutations, acting as dominant-negative, poses a challenge for the development of targeted therapies. In contrast to gene replacement, allele-specific silencing has the potential to treat these disorders, as it would convert the dominant-negative state into a clinically asymptomatic haploinsufficient state. Therefore, in the laboratory we aim at exploring targeted RNA interference (RNAi) approaches as a potential therapeutic approach for dominant COL6-RD. We have previously demonstrated the allele-specificity and efficacy of siRNA oligos to downregulate the expression of a mutant *COL6A3* transcript *in vitro* in patient-derived fibroblasts. In preparation of in vivo testing we have now extended our study to cells isolated from a mouse model of the disorder, which carries the most frequent dominant-negative mutation, a deletion of exon 16 on *Col6a3*. We transfected a series of small interfering RNA (siRNA) oligonucleotides into Col6a3del16+/- fibroblast cells, isolated from skin and muscle. To assay efficacy we used outcome measures such as unsaturated PCR, quantitative RT-PCR, and immunofluorescence. In addition, we performed in vivo electroporation of siRNA oligonucleotides in FDB muscles of Col6a3del16+/- mice. We found that siRNA oligonucleotides were effective in Col6a3del16+/- cells, to specifically knockdown the expression from the mutant allele and to restore the production of a collagen VI matrix in culture. This study provides further proof-of-concept of the use of RNAi as a potential treatment for COL6-RD.

151. Abstract Withdrawn

152. Tumor Inhibition by Using Chitosan:siRNA PDGFR-ß in Breast Cancer Model of Rat

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Platelet-derived-growth factors (PDGFs) may represent a novel target in human cancer because they regulate many cellular processes, including cell proliferation, transformation, apoptosis and angiogenesis. PDGF-D's exert their cellular effects by two receptors PDGFR-α and PDGFR-β. Moreover PDGF ligands and receptors are proto-oncogenes that can be activated by various types of alternatives in cancer cells. Breast cancer is the most common and fatal type of cancer. The PDGF pathway is essential in tumor angiogenesis. It is known that the expression of PDGF receptors is altered and upregulated in breast cancer. Delivery is very important for success in gene therapy. Among the different non-viral gene delivery system, chitosan has very useful properties as a gene carrier. In this study; we investigated the effect of chitosan:siRNA complexes on tumor angiogenesis in breast tumor models of rats. Firstly chitosan:siRNA complexes (1/5-1/20 rate) targeting to PDGFR-β were prepared and characterized *in vitro*. Transfection efficiency of these nanoplexes in breast cancer cells such MCF-7, MDA-MB-231 and MDA-MB-435 was assayed. In *in vitro* transfection studies, 54%-65% of PDGFR-β inhibition was measured. Then these chitosan:siRNA complexes were injected intratumorally to tumor bearing Sprague Dawley rats. After tumor reached to constant size, complexes (20/1) were injected (40µg siRNA) and tumor volumes were measured periodically. After sacrification of animals, tissue and blood samples were taken and investigated histologically and immuno-histochemically. Expression levels of PDGFR-β determined by ELISA, mRNA levels were measured by RT-PCR also. Nanoplexes having 207 ± 3.5 nm size and 14.2 mV surface charge were used in this study. Tumor volume of nanoplex treated rats decreased at 92.49% at the end of the experiment (28th day). After free siRNA treatment, however, tumor volume increased very slowly until 21 days (68.1 % of decreasing value) then went up fastly. RT-PCR, Western-blot studies and immuno-histochemical data showed similarity with tumor volume measurements. Similar IFN response with tumor (untreated) was obtained after nanoplex administration. In conclusion, chitosan can be complexed with siRNA targeting PDGFR-β and chitosan:siRNA PDGFR-β nanocomplexes may be useful alternative in the treatment of breast cancer. The receptors of PDGF may be also suitable target in breast cancer.

153. Third Generation Antisense Targeted to Double Homeobox Protein 4 (DUX4) Reduced DUX4 Expression and Improved Differentiation of FSHD Myoblasts

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant expression of double homeobox protein 4 (DUX4) gene at chromosome 4q35. To date there is no effective treatment for the disease. In the current study, we have evaluated selected Third Generation Antisense compounds (3GAs) targeted to DUX4 for