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P 52

The generation of animal models to design novel gene correction approaches for Pompe disease

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Background: Pompe disease is a lysosomal storage disease caused by autosomal recessive mutations in the acid-α-glucosidase (GAA) gene. The GAA deficiency results in glycogen accumulation in the lysosomes, often causing death. The only available therapy is enzyme replacement therapy. Several gene therapeutic approaches can be envisioned for this disorder including gene correction therapy. Unlike Pompe patients, available mouse models show high GAA plasma levels. We developed a mouse model having no/low plasma GAA activities using siRNA. In parallel, DNA binding agents are fused to restriction endonucleases to 1) introduce GAA-mutations resulting in a relevant Pompe animal model and 2) correct the mutated gene in this disease model.

Methods: siRNAs against GAA were delivered in fibroblasts using the cationic lipophilic compound SAINT. In BL6 mice, 50μg of SAINT ± siRNA was injected every day. GAA was determined using 4-MUG assay or mRNA levels. Triplex forming oligonucleotides fused to restriction endonucleases (MunI, scPvuII) and zinc finger nucleases were engineered to target the GAA gene. These so called meganucleases are supposed to introduce double strand breaks into the target gene and thereby increasing the frequency of homologous recombination with an appropriately modified gene fragment supplied together with the meganuclease.

Results: Delivery of siRNA decrease GAA activity over 60% in vitro, and with 45% after 14 days in plasma. No reduction of GAA mRNA or protein was found in tissues from siRNA+SAINT treated mice compared to control mice. Targeted nucleases were constructed and resulted in cleavage of target sequences.

Conclusion: Delivery of non-modified siRNA leads to a decrease in plasma GAA activity in vivo and serves as the basis for a clinically relevant Pompe model. This model will then be used to establish the power of engineered gene-specific meganucleases as a new tool in obtaining disease models.

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man cancer (1). Present treatments for GBM, such as surgery, gamma-irradiation, and chemotherapy are ineffective in eradicating the cancer, evidenced by poor prognosis for glioma patients with a mean survival time of less than 1 year after diagnosis. Hence, there is an urgent need to improve the efficacy of therapies for this fatal disease.

Method: Identification of the specific sequence by cDNA microarray followed by confirmation using real-time PCR. Cloning of sequence into a baculoviral vector to drive the expression of suicide gene thmidine kinase.Demonstration of in vitro efficacy using cell death assays in presence of ganciclovir and establishment of proof of concept using a mouse xenograft model for gliomas in vivo.

Results: HMGB2 was identified as one of the genes having low expression level in normal human astrocytes, but was significantly up-regulated in U251 glioblastoma cells by cDNA microarray analysis. Real-time PCR quantification confirmed 11 to 79 fold increase in HMBG2 expression in glioblastoma tissues vs normal human brain tissue. We cloned a 2kb 5′ upstream region of the HMGB2 gene. By progressive truncation of the 2kb fragment, we identified a 500bp fragment displaying very high transcriptional activity in glioblastoma cells, but a low activity in normal brain cells (primary neurons and normal human astrocytes). Baculoviral constructs with 500bp sequence driving the expression of the HSVtk gene were lethal to glioblastoma cells(cell survival ~18% after 48Hrs) in the presence of ganciclovir, whereas normal human astrocytes and neurons were not affected. We further confirmed the efficacy of the baculoviral vector in suppressing the growth of human Glioblastoma cells after intra-tumor injection in a mouse xenograft model.

Conclusion: We have demonstrated the identification of a novel 5′-upstream sequence of the HMGB2 gene which has a potential to be used as an efficient, tumor-selective promoter in targeted glioblastoma gene therapy.

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P 54

Adenovirus tropism modification on mesenchymal stem cells infection for in vivo visualization and cancer treatment

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