Overview

In utero gene therapy has been proposed as a viable treatment for a range of genetic diseases including cystic fibrosis (CF).

Potential advantages include targeting of expanding progenitor cell populations and reduced tissue and immunological barriers to gene transfer.

Published studies (Larsen et al., Lancet (2000), 349; 619-620) have suggested that transient expression of CFTR in utero is sufficient to rescue the fatal intestinal defect observed in S489X Cftr tm1UNC knockout mice.

We have replicated these studies using an identical adenoviral CFTR vector and sufficient numbers of mice to provide robust Kaplan-Meier survival data.

Results

Figure 1  Bio-distribution of fluorescent beads following intra-amniotic injection

To investigate the potential distribution of adenoviral vectors following intra-amniotic injection, 100 nm red fluorescent beads were injected into C57BL6 fetuses at days 14 (E14), 16 (E16) and 18 (E18) of gestation.

Effective delivery of virion sized beads to the developing lungs and intestines was only observed following injection at day 16 (E16) and day 18 (E18) of gestation.

E16 was selected as the delivery timepoint for future studies to ensure adequate delivery to CF related organs.

Figure 2  Luciferase expression following in utero delivery of AdLuc

To investigate gene expression following intra-amniotic injection of adenoviral vectors, C57BL6 fetuses were injected with between 10^6 and 10^8 pfu of the luciferase expression vector AdLuc at E16 and expression measured 48 hr later.

Dose dependent luciferase expression was observed in both the lungs and the intestines of treated fetuses.

Intra-amniotic injection of adenovirus at E16 results in robust gene expression in CF related organs.

Figure 3  hCFTR expression in S489X knockout mice

Litters resulting from heterozygote matings of S489X Cftr tm1UNC mice were injected with 10^7 or 10^8 pfu of the hCFTR expression vector Av1CF2 at E16 and expression of hCFTR mRNA was assayed 48 hr later.

Generation of fully mature and functional hCFTR by Av1CF2 was confirmed by western blotting (a) and 125I efflux analysis (b).

Robust hCFTR expression was detected in both the lungs (c) and intestines (d) of Cftr (-/-) mice at equivalent levels to (+/-) and (+/+). littersmates.

Intra-amniotic injection of Av1CF2 at E16 results in robust hCFTR expression in CF related organs in S489X Cftr tm1UNC mice.

Figure 4  Survival of S489X Cftr tm1UNC knockout mice after in utero gene therapy

Litters resulting from heterozygote matings of S489X Cftr tm1UNC mice were injected with 10^7 or 10^8 pfu of Av1CF2 at E16. Pregnancies ran to term and survival of Cftr (-/-) pups as well as (+/-) and (+/+). littersmates was monitored for 100 days after birth and compared with mice from untreated litters or litters that received 10^7 pfu of the control vector AdLacZ.

Without intervention, Cftr (-/-) mice die within several weeks of birth due to intestinal complications.

Only 16% of untreated Cftr (-/-) S489X Cftr tm1UNC mice survived to 100 days after birth.

No improvement in survival was observed in Cftr (-/-) S489X Cftr tm1UNC mice following intra-amniotic injection of Av1CF2.

Conclusions

Intra-amniotic injection of gene transfer agents at an appropriate gestational stage can mediate gene expression in organs appropriate for CF gene therapy.

However, transient expression of CFTR in utero does not correct the fatal intestinal defect in S489X Cftr tm1UNC mice.

In utero gene therapy remains a potentially valuable approach for the treatment of a range of genetic diseases.