Adenovirus-Mediated *In Utero* Expression of CFTR Does Not Improve Survival of CFTR Knockout Mice

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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

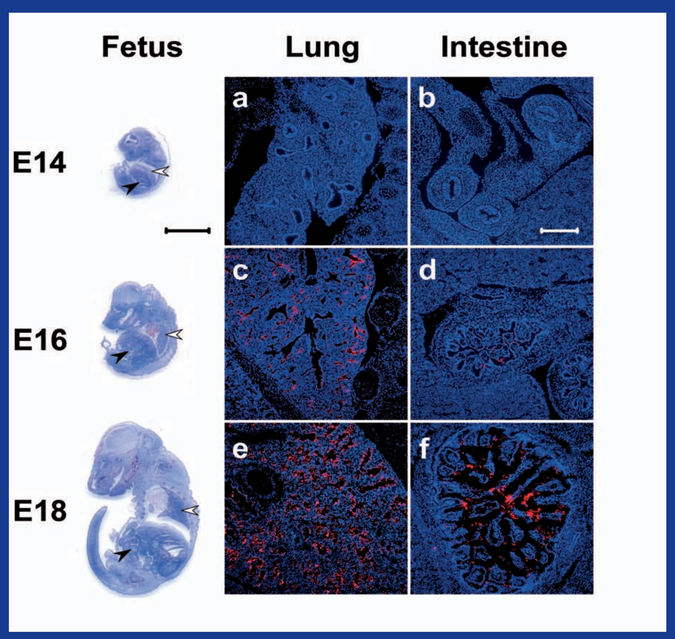

Figure 1 Bio-distribution of fluorescent beads following intra-amniotic injection

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

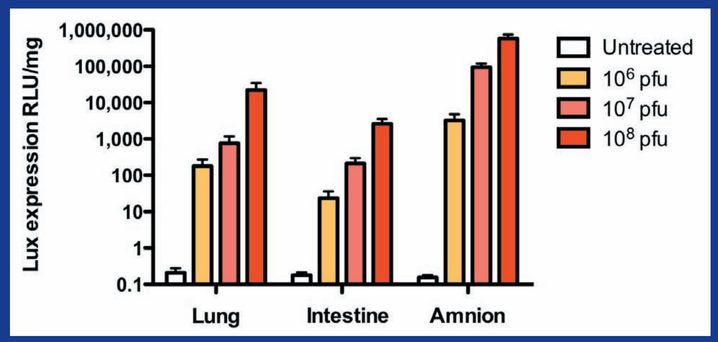

Figure 2 Luciferase expression following *in utero* delivery of AdLuc

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

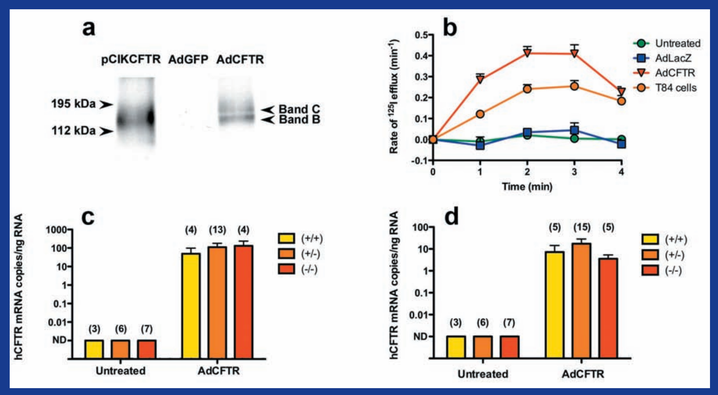

Figure 3 hCFTR expression in S489X knockout mice

Litters resulting from heterozygote matings of S489X Cftr^{tm1UNC} mice were injected with 10^8pfu 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 ^{125}I efflux analysis (b)

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

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

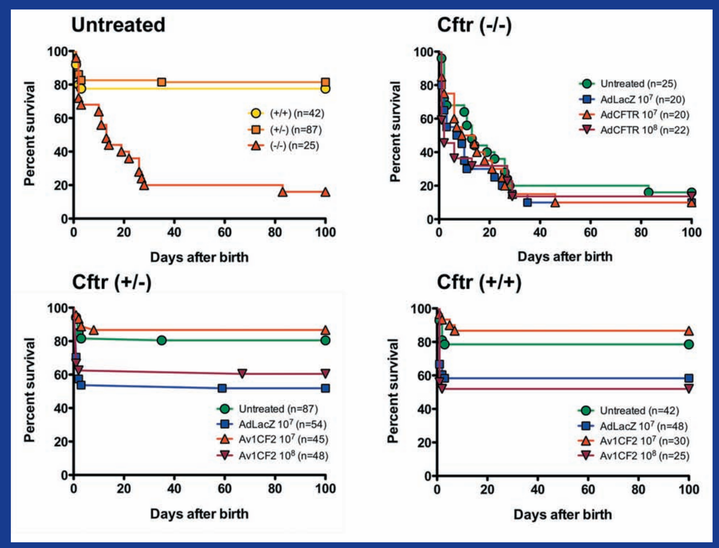

Figure 4 Survival of S489X Cftr^{tm1UNC} knockout mice after *in utero* gene therapy

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

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

Cftr (+/-) and (+/+) littermates demonstrate normal survival

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