Inflammation-Free shRNA Expression Vectors for Cystic Fibrosis Gene Therapy

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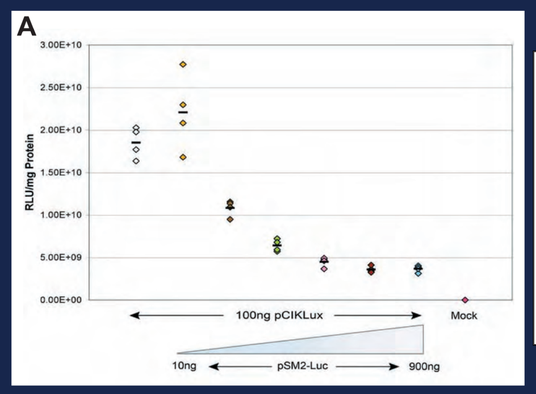
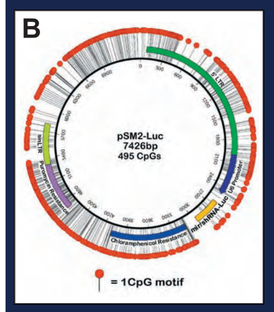
OVERVIEW

- The plasmid DNA (pDNA) component of non-viral vectors contains a high number of CpG sequences that cause inflammation and flu-like symptoms when administered *in vivo* in pre-clinical and clinical studies[1].
- We have recently developed CpG-free pDNA vectors for the treatment of Cystic Fibrosis (CF) lung disease. These have been shown to direct sustained, inflammation-free transgene expression when delivered to the lungs of animal models[2].
- The delivery of such vectors expressing the CFTR epithelial chloride channel to the lungs of CF patients is being evaluated clinically.
- However, CF lung pathology is also linked to excessive sodium absorption via the epithelial sodium channel ENaC. Here we describe the development of CpG-free pDNA vectors to express RNA interference (RNAi) molecules suitable for ENaC inhibition.

CONVENTIONAL shRNA EXPRESSION VECTORS

- Conventional RNA expression vectors utilise pol*III* promoters (such as U6) to express shRNA sequences. Processing of shRNA molecules is enhanced by encapsulating the shRNA within a miRNA backbone. Such expression vectors direct robust *in vitro* RNAi (A; ~80% knockdown of co-transfected luciferase expression p=0.021)



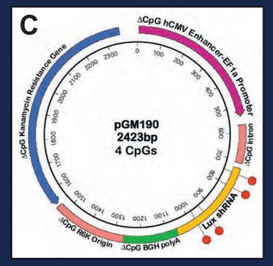


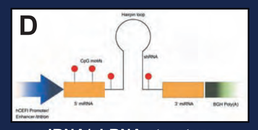
- However, conventional miRNA/shRNA expression vectors are rich in inflammatory CpG sequences (B), known to cause systemic inflammation and flu-like symptoms when administered *in vivo.*

References:
1. Alton *et al* (1999) Lancet 353, 947-954.
2. Hyde *et al* (2008) Nature Biotechnology 26, 549-51 (utilising CpG-free technology developed by Cayla-InvivoGen, Toulouse, France).

CpG MINIMISATION

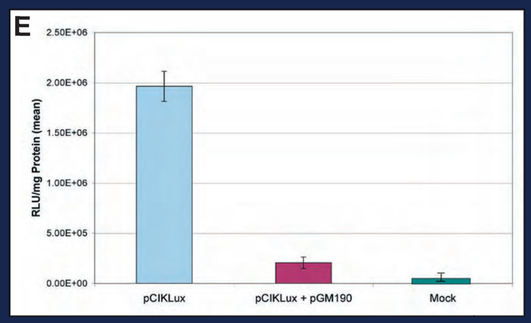
- To minimise the inflammatory CpG content we eliminated unnecessary elements within pSM2-Luc and replaced its pDNA backbone with our novel CpG-free form[2] to create pGM190 (C & D).
- In pGM190, transcription of the miRNA/shRNA expression cassette is under the control of the CpG-free pol*III* hCEFI enhancer/promoter element, known to express abundant and sustained lung expression after *in vivo* gene delivery[2].

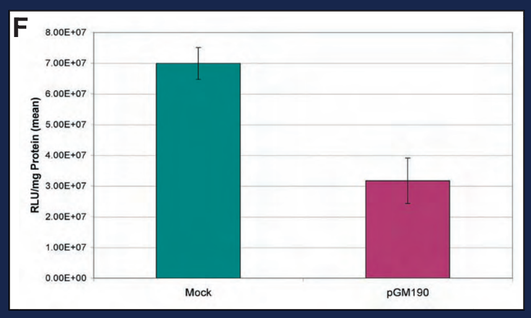




miRNA/shRNA structure

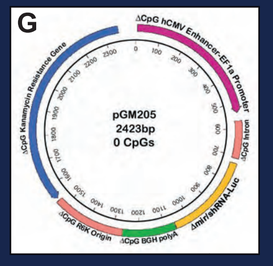
- pGM190 directs robust *in vitro* RNAi following co-transfection of the targeted gene (E; ~80% knockdown p=0.042) or of an endogenously expressed gene (F; ~60% knockdown p=0.025).

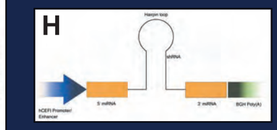




CpG ELIMINATION

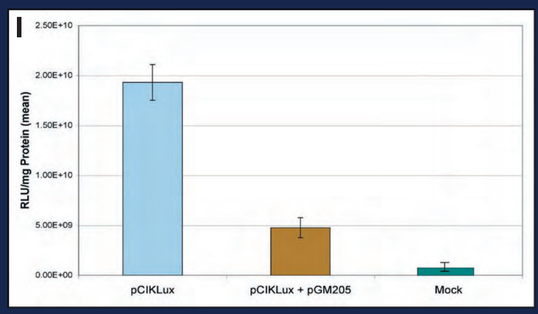
- As even a single pDNA CpG has been shown to direct *in vivo* inflammation[2], we further modified pGM190 to eliminate the 4 remaining CpGs from the miRNA backbone.
- Energy minimisation and secondary structure analyses were used to retain the predicted miRNA structure during CpG elimination. 6 nucleotide replacements were required to eliminate the 4 CpGs whilst retaining the desired secondary structure (G & H).





miRNA/shRNA structure

- pGM205 directs robust in vitro RNAi following co-transfection of the targeted gene (I; ~80% knockdown p=0.022).



CONCLUSIONS

- miRNA/shRNA sequences can direct efficient knockdown when under the transcriptional control of a pol*III* promoter.
- Depleting CpG motifs within the miRNA/shRNA does not appear to have a detrimental effect on knockdown compared to CpG-rich native versions.
- This system could be used to deliver CpG-free anti-ENaC miRNA/shRNA sequences within a pDNA vector as a treatment for CF lung disease.