GNAS R201C specific silencing in FD mice using liposome delivered dCas9

FD is a dominant, non-inherited bone disease characterized by high proliferation and osteogenesis disorder of bone marrow stromal cells (BMSCs).
The pathogenesis of FD remains unclear, and thus there is still no cure. in this work we designed a liposome-delivered dCas9 based treatment aimed at specifically silencing the expression of the mutated allele to restore the healthy phenotype.

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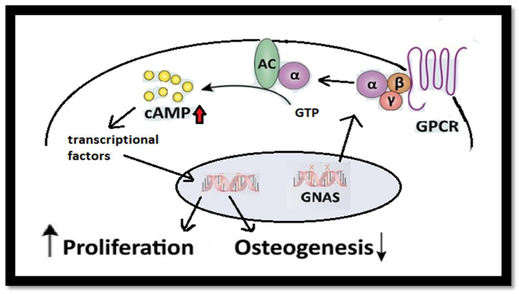
BACKGROUND

Fibrous Dysplasia is a skeletal disease caused by non-inherited missense mutations in the gene GNAS which encodes the α subunit of the stimulatory G protein. Mutations occour postzigotically in BMSCs.

2 Point mutation in exon 8: Arg201 is substituted by a Cys (R201C) or an His (R201H)
R201C is more frequent; is the consequence of a methylation-deamination sequence affecting the cytosine in the CpG dinucleotide of the 201 codon (5'-CGT-3')

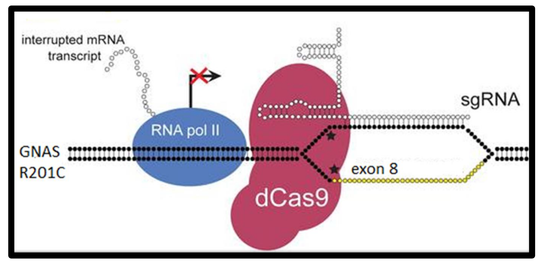
R201C is a gain of function mutation : decreased GTPasic activity of Gsα mutated protein leads to a constitutive activation that cause an overstimulation of adenylyl cyclase, and excess cAMP production.



AIM AND STRATEGY

AIM: specific silencing of mutated allele.

STRATEGY: dCas9 target GNAS R201C causing steric block that halts transcript elongation by RNA polymerase.

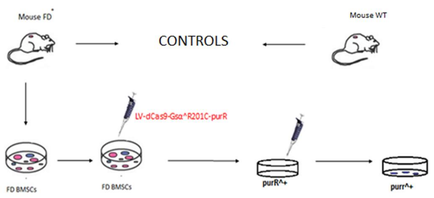


Ctg ctt cgc tgc tgt gtc cgt acc tct gg aat GNAS R201C forward strand

5' cgc tgc tgt gtc cgt acc tc sgRNA

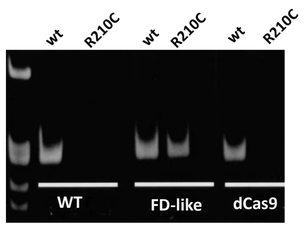
IN VITRO

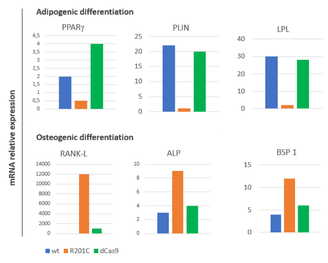
dCas9 and sgRNA lentiviral expression in BMSCs R201C



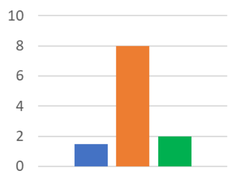
Results:

After LV transduction only wt Gsα was expressed

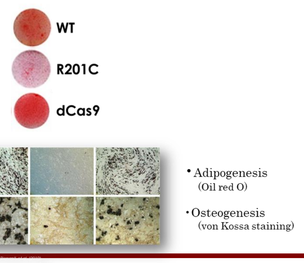




cAMP assay



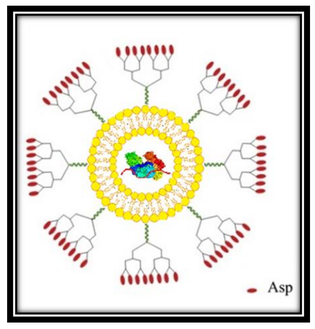
Calcium deposition



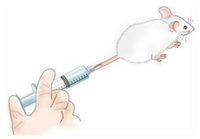
- Adipogenesis (Oil red O)
- Osteogenesis (von Kossa staining)

IN VIVO

dCas9 asp-coated liposome delivery



High bone delivery efficiency due to Asp affinity for HA

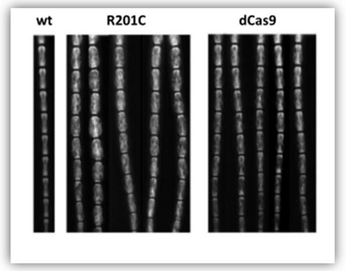


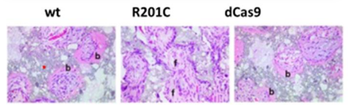
Mice were treated every 6 days for 60 days, since early symptoms appear

Administration way:
Tail vein injection

Therapy efficacy was evaluated by histological analysis and radiography

Results:





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PITFALL AND SOLUTIONS

Development of a more effective and permanent treatment based on dCas9 to reduce treatment frequency

Construction of a liposome with higher affinity for bone cells

Design of sgRNA for all mutations that cause FD

BUDGET

Mice C57BL/6	€ 600,00
Mice FD like	---
cAMP assay	€ 299,00
Crispr/cas9 kit	€ 300,00
PCR Kit	€ 300,00
Lentivirus	€ 500,00
dCas9	€ 150,00
sgRNA	€ 200,00
Puromycin	€ 140,00
Stabulation costs	€ 3000,00 per year
Salaries for researches:	€ 80.000,00 per year

TOTAL COST: € 85.500