“The potential use of RNA interference to down-regulate the BMP-antagonist Noggin and increase bone formation during distraction osteogenesis.”

Ana Cristina F. Bassit
Marie-Hélène Gaumond
Pierre Moffatt
Reggie Hamdy
Distraction Osteogenesis (DO)

• Unique surgical technique that stimulates bone formation, most frequently used to promote limb lengthening through slow and progressive distraction after osteotomy.

• The great challenge is to reduce the consolidation phase and prevent the complications related to the maintenance of the external fixator for a long period.
Bone morphogenetic proteins - BMPs

1965 – Urist demonstrated that the implantation of demineralized bone matrix could induce ectopic bone formation.

Bone morphogenetic proteins (BMPs) were identified as signaling molecules that participate in the skeleton development during embryonic phase.

BMPs also play a key role in bone healing and have been used as potent osteoinductive growth factors.
Bone morphogenetic proteins - BMPs

BMPs decrease time for fracture healing and increase bone regeneration, but the use of exogenous BMPs is still controversial.

We considered manipulating the BMP-antagonist Noggin through RNA silencing to increase endogenous BMPs levels.
BMP antagonist
Noggin
## Alignment

![Alignment screenshot](image)

### Clipping View

- **Query 1**:
  
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### Cloning View

- **Cloning View**: 
  
  ![Cloning View](image)

### Cloning View Details

- **Runs**:
  
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- **Query Sequence**:
  
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  GCGGCCGCGCCTCCCAAGTAGAGGCGCggggggAGTTGTGACCACACTCGTGCCGCTCTTC
  ```

- **Sbjct Sequence**:
  
  ```
  GCGGCCGCGCCTCCCAAGTAGAGGCGCggggggGAATTGCGACCAACACTCGTGCCGCTCTTC
  ```

### Reference

- **Mus musculus noggin (Nog), mRNA**
  
  Sequence ID: ref|NM_008711.2| Length: 1922 Number of Matches: 1

### Additional Information

- **Alignment Details**:
  
  - **GenBank**
  - **Graphics**
Max score = Total score
Ident. = 97%
Sigma Mission shRNA

#1 – (165-185) shRNA – N185 – GD # 609
#2 – (420-440) shRNA – N420 – GD # 610
#3 – (137-157) shRNA – N157 – GD # 611
#4 – (78-98) shRNA – N98 – GD # 612
#5 – (354-374) shRNA – N374 – GD # 613

noggin NM 008711 (699bp)
GD610 (pLKO.1-puro-Noggin TRCN0000066294 (N420))

7084 bp

ampi

puropart of Env GP160 glycoprotein

TRCN0000066294 (N420)

pLKO-REV

pLKO-FWD

U6

puroFWD

puro

puro-REV

hPGK

partial U3

SIN/3'-LTR

U5'

SV40pA

bla promoter

5'GAG D3rdG

Psi

SD

HIV 5'-LTR

RSV

pUCori

ampi

SD

Psi
• These vectors also encoded a drug resistance gene inactivating puromycin for posterior selection of the cells

• After bacteria amplification, plasmids were purified (QIAGEN® Plasmid Purification kit).
The production of lentiviruses expressing the shRNAs was realized by transfection of HEK cells, with X-tremeGene 9 DNA Transfection Reagent - Roche®.

Seventy two hours after transfection, the media was collected and lentiviral particles were concentrated by ultracentrifugation.
Infection with lentiviruses

UMR106 rat osteosarcoma cells were seeded in 12-well plates and infected with the Noggin and nontarget shRNA lentiviruses. Followed by amplification
Selection with puromycin

Added 2.5 µg/mL of puromycin to media.
Cells and conditioned media were collected and processed for qRT-PCR and Western blotting to analyze Noggin mRNA expression and protein production and secretion.
The lentiviruses expressing shRNA 609 and 610 caused a decrease of approximately 50\% of Noggin gene expression.
Noggin gene expression
Protein Expression

• Western blotting analysis showed an approximate 55% decrease in secretion of Noggin in the media of cells infected with shRNA 609 and 610.

• Also, Noggin cellular expression was reduced to less than one fifth as compared to NT-infected cells.
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Ponceau 2013-09-30

Detection 40 min exposition 2013-10-01

Detection 1 hour exposition 2013-10-01
Detection 20 min exposition 2013-10-23

Ponceau 2013-10-21

NT     609   610   610     NT    609    610    610

Media

Cells

6.25 µl

25 µl

Detection 40 min exposition 2013-10-23

Ponceau 2013-10-21

NT     609   610   610     NT    609    610    610

Media

Cells

175

80

58

46

30

25

17

7

6.25 µl

25 µl
Future research and clinical application

- Development of a DO external fixator for rats.

- In vivo studies: injection of shRNA will be done at the distraction site.

- Use of nanoparticles as an alternative siRNA delivery system.
Thank you!