Potential Target for Rare Dwarfism

Investigation of Creld2 and its action in cartilage and skeletal development

Casey Broadbent /140200537 / biomedical sciences/ C.Broadbent1@ncl.ac.uk / supervisor- Prof Michael Briggs

Background

A rare short limb dwarfism multiple epiphyseal dysplasia (MED) is characterised by growth plate abnormalities where cartilage is replaced by bone. It results from the accumulation and inadequate degradation of mutant protein that leads to increased cellular stress. Creld is thought to play a role in protein folding and trafficking due to increased levels in MED[1]. Here it potentially plays a role in combating the cellular stress.

Aims

To determine the role of *Creld2* in cartilage and skeletal development by studying a cartilage-specific *Creld2* knockout mouse model.

Methods

- Longditudinal X-ray analysis to measure long bones
- Hematoxylin and Eosin (H&E) staining to analyse the cartilage growth plate structure
- Immunohistochemistry to analyse the expression of the abundant cartilage protein Collagen X

Results

Figure 1. Bone length measurements from x-ray images comparing wildtype and knockout mice. (*=p<0.05)

Creld2 cartilage specific knockout mice display shorter long bones as well as a shorter skull when compared with wildtype controls.

		Average tibia length (mm)	Average femur length (mm)	Skull length (mm)
3 week females	*Creld2* wildtype	12.74 ± 0.47	8.09 ± 0.30	21.11± 0.63
	Creld2 knockout	11.61± 0.82*	7.09± 0.64*	19.73± 0.97*

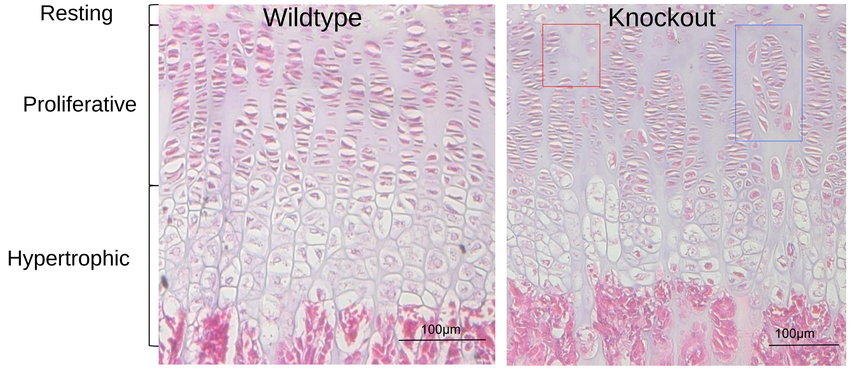

Figure 2. h&e staining of tibial growth plates at 3 weeks.

Knockout mice display a disrupted growth plate as there are gaps in the matrix (highlighted by the red box) and cells are not the correct morphology or arranging into ordered columns (highlighted by the blue rectangle).

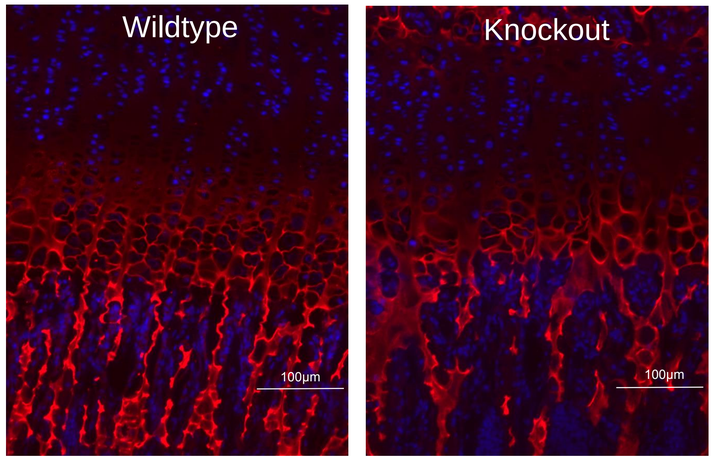

Figure 3. Immunohistochemical staining for the cartilage protein collagen X (Nuclei=blue (DAPI), Collagen X = red (secondary antibody = Alexa Fluor 594)

The cartilage growth plates in knockout mice appear to have reduced collagen X in the hypertrophic zone

Discussion

- As shown from **figure 1**, there is a reduction in the length of both the tibia and femur aswell as the skull. A novel role for *Creld2* in bone formation and elongation.
- An abnormal growth plate is observed in **figure 2** in the knockout mouse. The gaps and disorganisation compared with the columnar structure in the control demonstrates that *creld2* plays a role within growth plate and subsequently bone formation.
- As shown by **Figure 3** collagen X is reduced in the hypertrophic zone of the knockout compared with the wildtype model. This suggests that in the absence of *Creld2* there is extracellular matrix disruption leading to a loss of structural support in connective tissue. This suggests that *Creld2* plays an important role in either the production, folding or secretion of the extracellular collagen X

Conclusions

- When *Creld2* is knocked out in cartilage, mice exhibit irregular growth plate development, affecting cartilage and bone length
- **What next?**
- To overexpress *Creld2* in attempt to alleviate the cellular stress associated with MED in order to determine its therapeutic potential in this disease.
- Analyse the trancription and translation of proteins in cartilage cells following the ablation of *Creld2* to determine its precise role in cartilage.

Acknowledgments

Thanks to Professor Michael Briggs and Ella Dennis for supervising my project

References

1. Hartley C. L. et al. . Armet/Manf and Creld2 are components of a specialized ER stress response provoked by inappropriate formation of disulphide bonds: implications for genetic skeletal diseases. Hum. Mol. Genet. 22, 5262–5275 (2013).

Newcastle University