MPS II models for the study of joint and bone pathophysiology developed by CRISPR/Cas9 technology

Emilio Vaena [1], Maximiliano E. Ormazabal [2], Romina Ceci [1], Procopio Domingo [1], Alvarez Cecilia [1], Paula A. Rozenfeld [1], Andrea Dardis [2]

[1]*Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP, CONICET - UNLP), La Plata, Argentina*

[2]*Regional Coordinator Centre for Rare Diseases, Academic Hospital of Udine, Udine, Italy.*

ERNDIM 2025 SYMPOSIUM

INTRODUCTION

- Hunter Syndrome (Mucopolysaccharidosis Type II) is caused by the deficiency in the lysosomal enzyme iduronate 2-sulphatase (IDS).
- The disorder compromises multiple tissues. In bones particularly causing coarse facial features, skeletal deformities, thoracolumbar kyphosis, macroglosia, macrocephaly.
- Available treatments only show effects at visceral level, with limited effects in bones and joints.
- Current in vitro and in vivo models of MPS II provide limited information about the molecular basis of the disorder, however pathogenic mechanisms involved in bone and joints pathophysiology are still unknown

AIM

- To develop new in vitro cellular models of MPS II bone and joints tissues editing the IDS gene through CRISPR/Cas9 technology.

METHODS AND RESULTS

CRISPR/Cas9 IDS gene editing

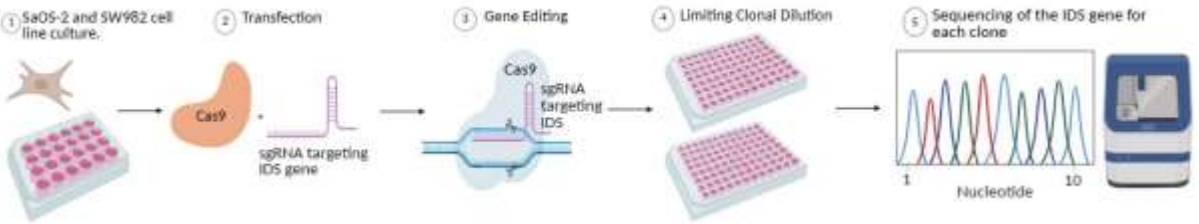
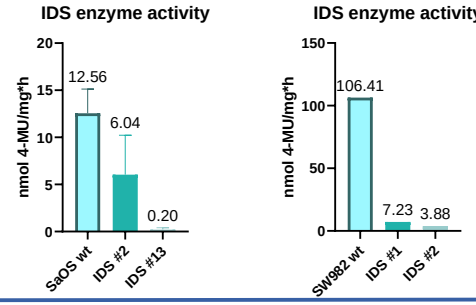


Fig. 1: Genome editing workflow in the SaOS-2 and SW982 continuous cell line. We obtained different clones with IDS sequence alterations

IDS Enzyme activity

Fig. 2: Scheme of the IDS enzyme activity assay by fluorimetric method based in the use of 4-MU-IdoUA(2S) performed in SaOS-2 and SW982 wild type and edited clones.



Pro-inflammatory state

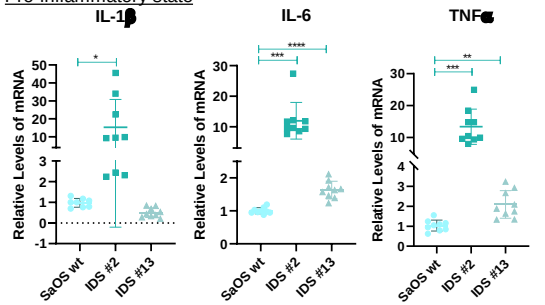


Fig. 3: Evaluation of mRNA expression levels of IL-1β, IL-6, TNFα through qPCR of SaOS wild type and clones. p < 0,0005 t-test

Apoptosis

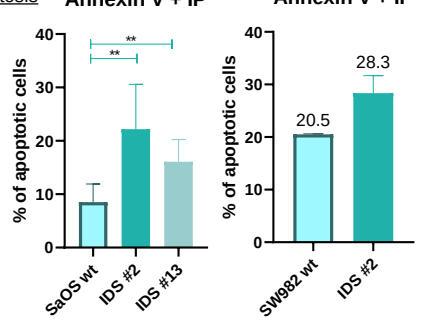
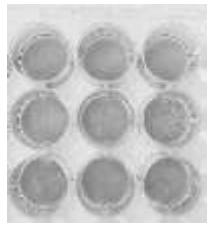


Fig. 4: Evaluation of alterations in apoptosis levels by Anexin V Alexa-Fluor 647 through flow cytometry of SaOS, SW982 wild type and clones. p < 0,0005 t-test

Alkaline Phospatase



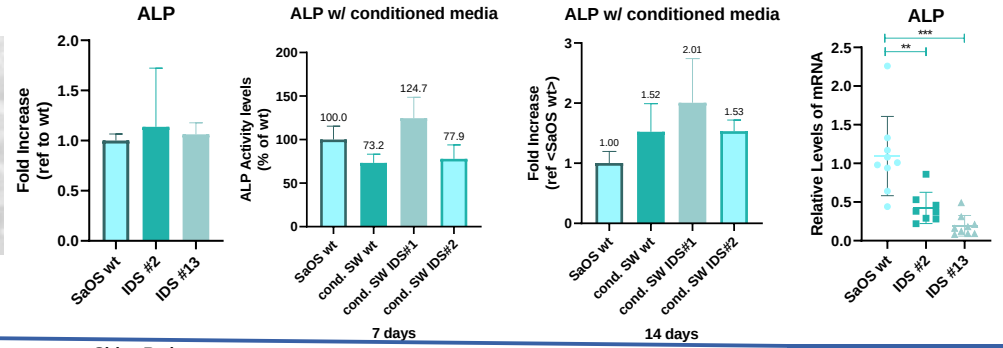


Fig. 5: Evaluation of ALP activity and expression levels by ALP staining through use of NBT/BCIP and mRNA by qPCR analysis. p < 0,0005 t-test

Signaling Pathways

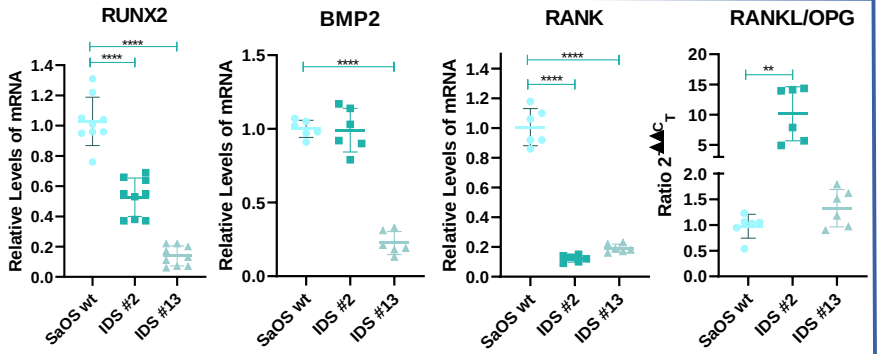


Fig. 6: : Evaluation of mRNA expression levels of RUNX2, BMP2, RANK, RANKL and OPG through qPCR of SaOS wild type and clones. p < 0,0005 t-test

Sirius Red

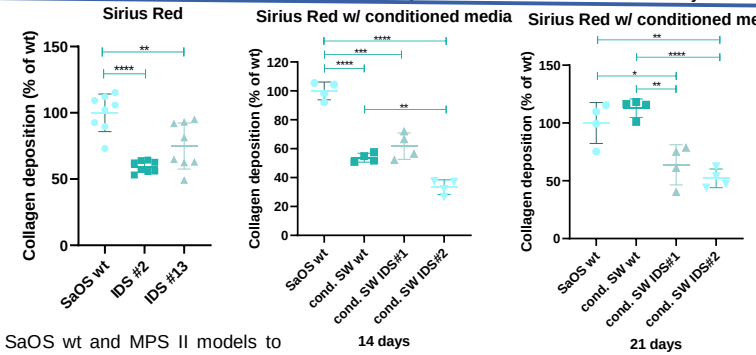


Fig. 7: Evaluation of SaOS wt and MPS II models to produce a mineralized matrix through collagen deposition analysis through Sirius Red staining. p < 0,0005 t-test

CONCLUSIONS

- We generated cells lines models to study some aspects of bone and joint pathophysiology.
- In the osteoblasts model we observed an increase in inflammatory cytokines and apoptosis levels.
- Alterations in mRNA levels of molecules involved in the balance of bone cells population.
- ALP mRNA levels are decreased in osteoblast models, although there no alterations in the activity.
- Collagen deposition its decreased in MPS II bone models compared to wild type cells.
- Synovial fibroblast model of MPS doesn't show alterations in apoptosis levels but have an impact in capacity of osteoblast wild type cells to produce a mineralized matrix