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MMP9 deficiency protects against trauma-induced heterotopic ossification

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INTRODUCTION

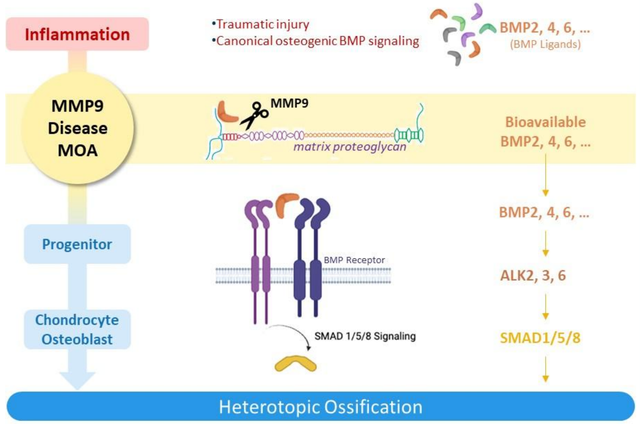
Non-hereditary heterotopic ossification (NHHO) is a condition of progressive formation of heterotopic bone (HO) in soft tissues, in the absence of a genetic mutation, often following trauma. Spinal cord and traumatic brain injuries (neurogenic HO), hip arthroplasty, accidents, blast injuries and severe burns can give rise to the formation of extraskeletal bone in joints, muscles, or tendons. HO can cause pain around the ossification site, loss of joint mobility and subsequently loss of function. There are no approved therapies to treat NHHO and surgical resection often results in recurrence. Nonclinical studies in model systems and analysis of human HO tissues have revealed signaling by canonical bone morphogenic proteins (BMPs) as regulators of the aberrant progenitor differentiation that leads to HO. Matrix Metalloproteinase-9 (MMP9) is highly expressed in HO lesions and was recently characterized as a critical regulator of HO in fibroplasia ossificans progressiva (FOP) (Lounev et al. 2024), in part via generation of bioavailable activin A from local extracellular matrix reservoirs, a mechanism that is also applicable to canonical BMP signaling.

The potential benefit of MMP9 inhibition as a therapeutic strategy in NHHO was evaluated using MMP9-deficient mice (MMP9 KO), in a burn-tenotomy model of NHHO.

PROPOSED MECHANISM OF ACTION

- Strong induction of MMP9 expression and activity with trauma, both systemically and locally at the site of HO formation, vs limited expression in healthy tissues
- Sustained MMP9 expression is associated with formation of HO

MMP9 GENERATES BIOAVAILABLE BMP LIGANDS TO PROMOTE HO



METHOD

Based on the burn/tenotomy mouse model of HO developed by Peterson et al. (2015).

WT: wild-type mouse strain
JAX #019019
KO: MMP9-deficient mouse strain
JAX #007084

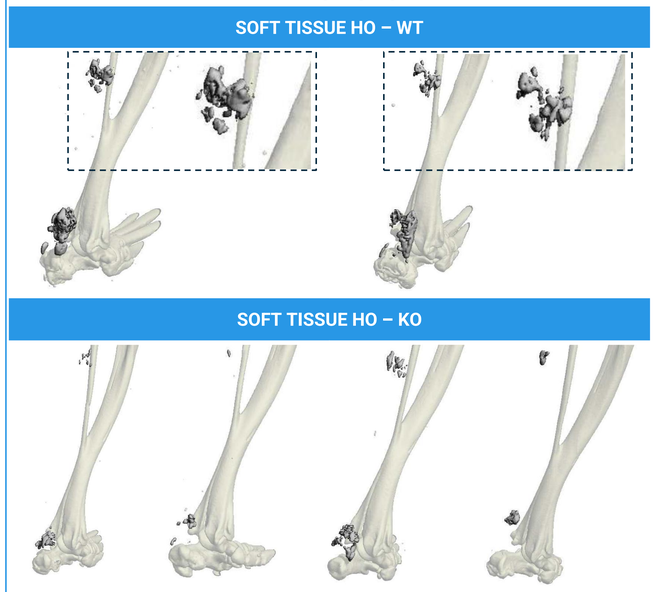
On day 1, ten mice per group (WT and KO) underwent Achilles tenotomy in the left hind limb by sharp dissection of the tendon at the midpoint followed by a dorsal partial thickness burn injury with an aluminum block heated to 60°C to induce HO.

After 62 days, the hind limbs were harvested, scanned using high-resolution micro-Computed Tomography (micro-CT), and analyzed for volume of HO using 3D morphometry.

Data were analyzed and visualized using Prism software. For HO volume assessment by micro-CT, the unpaired t test with Welch's correction was performed.

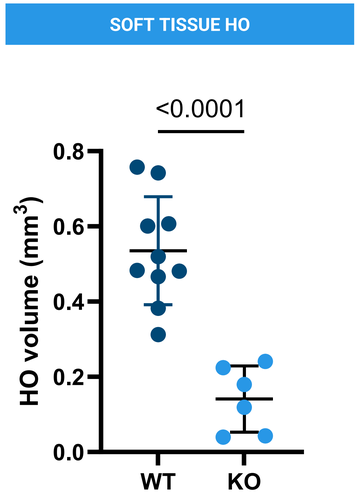
RESULTS

HO formation 62 days post burn/tenotomy injury in mice



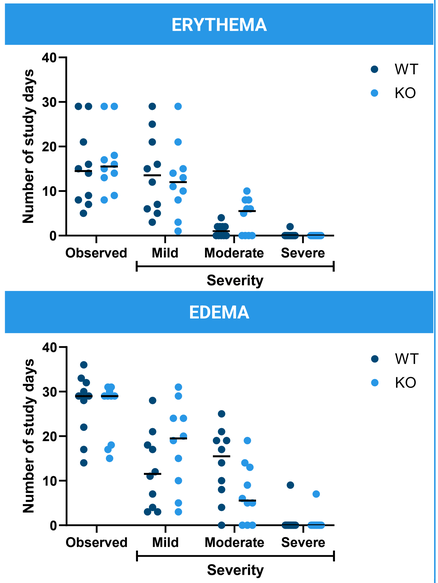
Representative images

MMP9 deficiency protects against formation of HO in mice



Scatter dot plot, line at mean ± SD
HO: heterotopic ossification
KO: MMP9-deficient mice
WT: wild-type control mice

Absence of MMP9 did not impair the healing process in mice



Scatter dot plot, line at median

DISCUSSION & CONCLUSIONS

Imaging of limbs from control WT mice revealed multiple foci of HO in soft tissue above and below the tenotomy site.

Mice deficient in MMP9 (KO) were significantly protected against formation of HO as compared to the controls ($p<0.0001$).

In addition, mice deficient in MMP9 recovered similarly from the burn injury and tenotomy to control WT mice, indicating that absence of MMP9 did not impair healing.

These data support the potential therapeutic benefit of MMP9 inhibition in NHHO.

A clinical trial with the MMP9 inhibitory antibody andecaliximab is currently enrolling patients with spinal cord injury at risk of developing HO.

REFERENCES

Lounev V, Groppe JC, Brewer N, et al. Matrix metalloproteinase-9 deficiency confers resilience in fibrodysplasia ossificans progressive in man and mice. *J Bone Miner Res.* 2024; 39(4): 382-98.

Peterson JR, Agarwal S, Brownley RC, et al. Direct mouse trauma/burn model of heterotopic ossification. *J Vis Exp.* 2015;(102):52880.

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