Midkine is involved in the pathogenesis of impaired osteoporotic fracture healing after ovariectomy in mice

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Objectives

Clinical data demonstrated significantly impaired bone regeneration in postmenopausal osteoporotic patients [1]. The molecular mechanisms behind that are still unclear. Therefore, there is a high clinical need for new treatment strategies.

One promising drug target molecule is the heparin-binding growth- and differentiation factor Midkine (Mdk), because:

- Mdk is supposed to be a negative regulator of bone formation [2]
- Mdk negatively affects Wnt-signaling and therefore osteogenic differentiation in osteoblasts [3,4]
- Antagonizing systemic Mdk accelerated bony callus formation during fracture healing [4]
- Mdk is an estrogen responsive gene with increased expression in the postmenopausal, diabetic kidney [5]

Is Mdk involved in delayed osteoporotic fracture healing?

Methods

Animal model: 3-months-old female wildtype mice (C57BL/6J)

Surgery: Bilateral ovariectomy (Fig. 1A); 4 weeks later: standardized femur osteotomy stabilized with an external fixator (Fig. 1B, C)

Treatment: Injections with 25 mg/kg BW Mdk-antibody (Mdk-Ab) or vehicle 2x/week for 3 weeks

Analyses: 3-point-bending test (Fig. 1D) and µCT (Fig. 1E) at day 23; histomorphometry and immunohistochemistry at day 10 and 23; Mdk serum ELISA at day 3,10 and 23.

Statistics: Kruskal-Wallis test with Dunn's post hoc (n=6-7; *p<0.05).

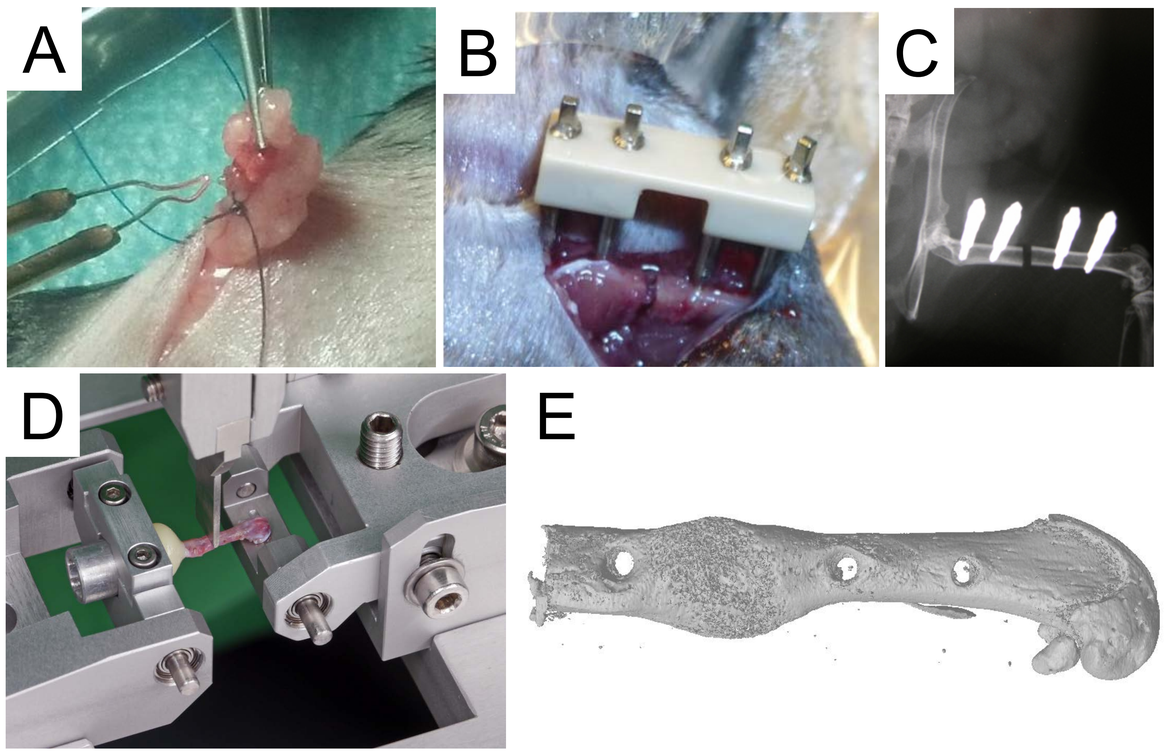
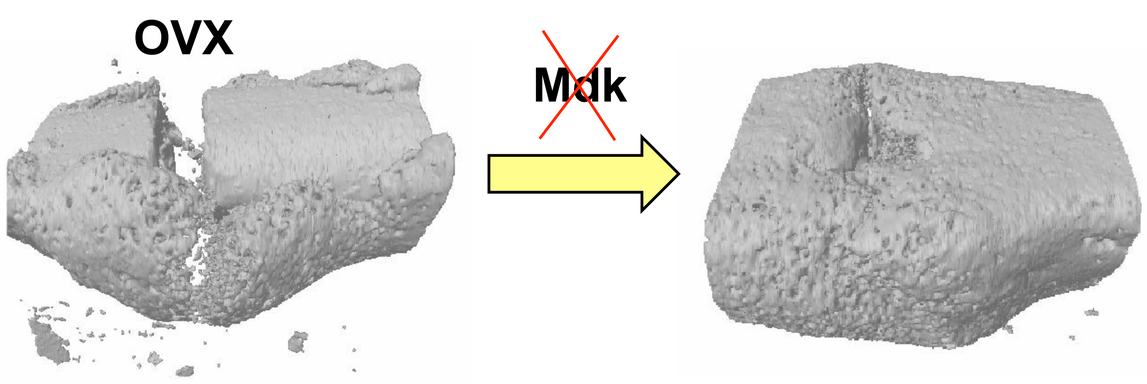


Fig. 1: A) *Ovariectomy of female mouse* ***B)*** *Mouse with an external fixator at the right femur directly after sawing of the osteotomy* ***C)*** *X-ray of a mouse with a femoral fracture stabilized by an external fixator* ***D)*** *Non-destructive 3-point bending test* ***E)*** *3D µCT reconstruction of a fractured femur*

Conclusions

- Mdk is involved in OVX-induced compromised fracture healing
- Accelerated healing after Mdk-Ab treatment
- Increased bone mass after Mdk-Ab treatment (callus and skeleton)



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The authors declare that no conflicts of interest exist.

Results

Increased Mdk serum levels after fracture in OVX mice (Table 1):

- fracture-induced increase of Mdk in the serum of sham-operated mice at day 3
- significantly higher and prolonged expression of Mdk in the serum of OVX mice
- significantly decreased Mdk serum levels after Mdk-Ab treatment

	treatment			
	sham		OVX	
days after operation	vehicle	Mdk-Ab	vehicle	Mdk-Ab
d0	n.d.		n.d.	
d3	38.6 ± 44.6	15.1 ± 33.9	67.9 ± 45.7	37.8 ± 43.6
d10	n.d.	n.d.	61.7 ± 43.6[a]	n.d.[b]
d23	n.d.	n.d.	31.0 ± 28.2	n.d.

Table 1: *Mdk serum levels in pg/ml during bone healing.*

Antagonizing Mdk abolished OVX-induced impaired healing:

- OVX compromised fracture healing by decreased biomechanical competence and bone formation in the fracture callus
- accelerated fracture healing after Mdk-Ab treatment in OVX mice
- beta-catenin expression is regulated by OVX (🡻) and Mdk-Ab (🡹)

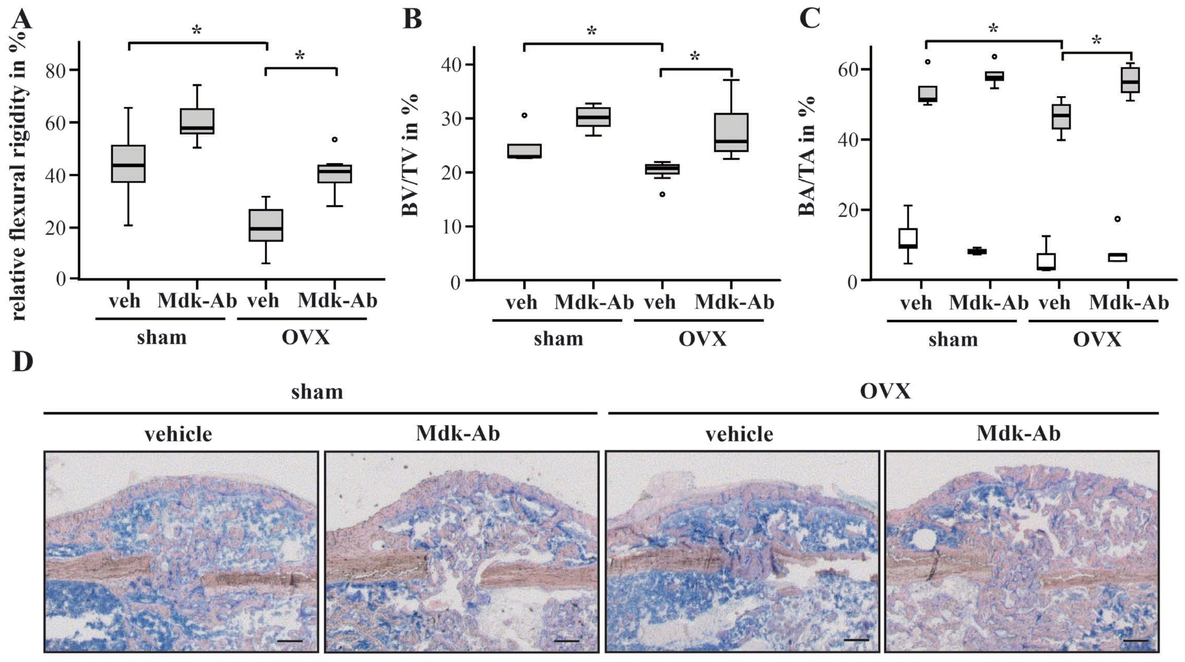

Fig. 2: A) *Biomechanical competence of the fracture callus relative to the intact femur, day 23* ***B)*** *µCT analysis: bone volume to tissue volume ratio, day 23* ***C)*** *Histology: Relative amount of bone in the fracture callus, white bars: day 10; grey bars: day 23.* ***D)*** *Callus sections from day 23, stained with Giemsa.*

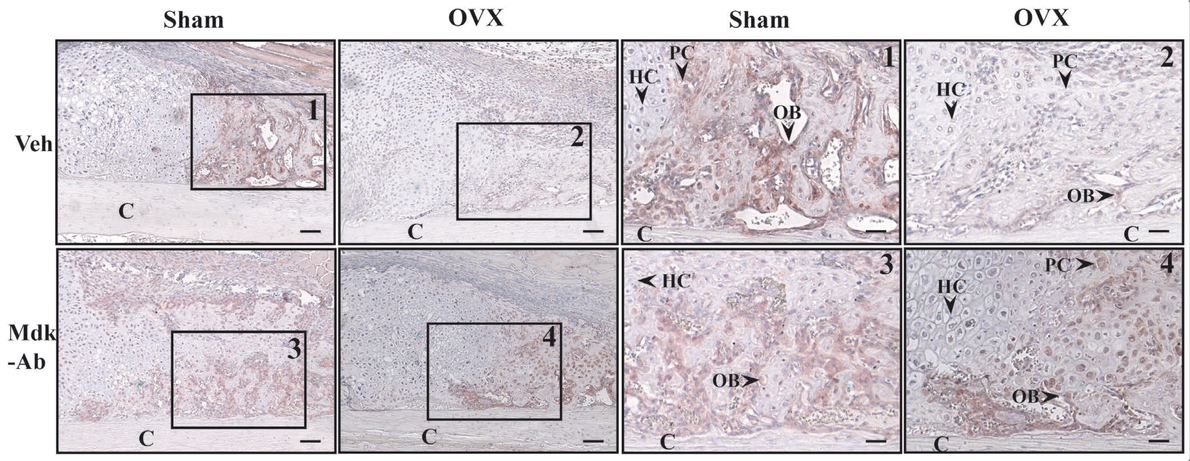

Fig. 3*: Immunohistochemical staining for beta-catenin in the fracture callus at day 10.*

Increased bone mass in the intact skeleton after Mdk-Ab treatment:

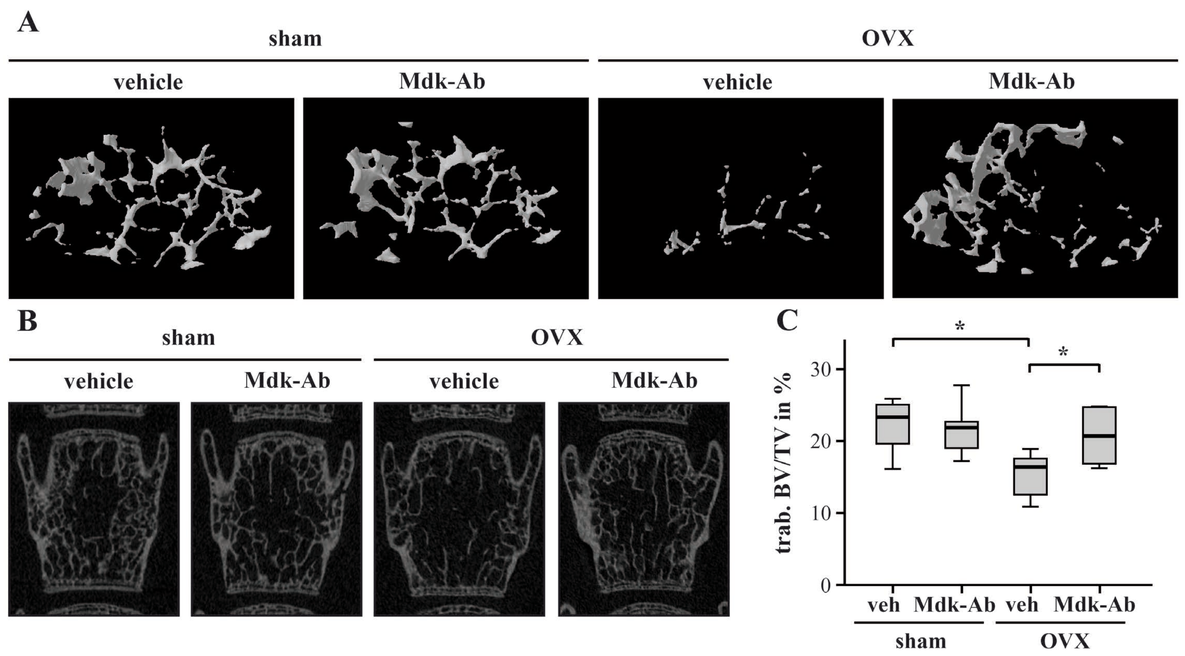

Fig. 4*:* ***A)*** *µCT 3D recons from the intact femur metaphysis B) µCT slices from the vertebral body L4* ***C)*** *Trabecular bone volume of L4; all from day 23.*