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

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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).

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?

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

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

<table>
<thead>
<tr>
<th>treatment</th>
<th>sham</th>
<th>OVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>days after operation</td>
<td>vehicle</td>
<td>Mdk-Ab</td>
</tr>
<tr>
<td>d0</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>d3</td>
<td>38±44.6</td>
<td>15.1±33.9</td>
</tr>
<tr>
<td>d10</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>d23</td>
<td>n.d.</td>
<td>n.d.</td>
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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 (ê) and Mdk-Ab (é)

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)

References


The authors declare that no conflicts of interest exist.