

# Elimination of Mitochondria Related Bim/Bid Proteins Reduces Chondrocyte Apoptosis after Mechanical Injury

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## INTRODUCTION:

Chondrocyte apoptosis following blunt mechanical injury is a well-known risk factor for the onset of posttraumatic osteoarthritis (PTOA) [1,2,4,6,7,8,9,12]. Recent studies suggest that an inhibition of nitric oxide can reduce apoptosis after trauma injury, but the molecular mechanism by which this inhibition of programmed cell death takes place is unclear [10]. Apoptotic death of mammalian cells is known to proceed by at least two complex pathways: Fas ligand (utilizing caspases 3, 6, 7, 8) pathway and mitochondrial or Bcl-2 mediated pathways (Bcl-2, Bcl-xL, Bax/Bak, Bim, and Bid) [3]. Bim is the primary mediator for Bcl-2 related cytokine withdrawal and deregulation of calcium flux where Bid is the key regulator of mitochondrial pathway for Fas related apoptosis through caspase-8 activation (Figure 1C). To fully understand how the mechanism by which chondrocytes undergo apoptosis following tissue injury is important to determine the role of Bim and Bid proteins in the process. To further illustrate the role of the mitochondria in chondrocyte apoptosis following mechanical injury, we have utilized a novel cartilage injury model and Bim/Bid double knockout mice. We hypothesized that the deletion of Bim/Bid in mice will prevent chondrocyte apoptosis following mechanical injury.

## METHODS:

**Mouse injury model:** Fourteen Bim/Bid double knockout (C57BL/6 Bim<sup>-/-</sup>Bid<sup>-/-</sup>) and fourteen matching wild-type (C57BL/6, Jackson Lab, Bar Harbor, ME) were included in this study [3]. The animals were maintained in the animal facility for one week before surgery using established protocols approved by the IACUC. All the mice were 7-weeks-old at the time of surgery. The xiphoid was exposed through a 1 cm incision centered over the distal end of the mouse sternum. A modified Kelly clamp was used to apply a crushing force of approximately 22 MPa to the exposed xiphoid. The clamp was applied three times (two minutes each time) with a one-minute pause between clamping (Figure 1A, 1B). The animals were sacrificed at 48 hours and the xiphoid harvested. **Histology, TUNEL and Caspase 3:** Cell matrix damage was determined using H&E and safranin-O/fast green, while apoptosis was determined using TUNEL, and activated caspase-3 staining [2,3,5]. Xiphoid cartilage was fixed and in paraffin and sectioned into 5µm sections. The total numbers of nuclei were determined by counter-staining with propidium iodide (PI). Images of the fluorescent specimens were captured with a CCD camera. Percentage of TUNEL+ (versus PI+) cells was analyzed using Image J. Adjacent cartilage specimens were stained with H&E, Safranin-O/fast-green and activated caspase 3 to confirm the TUNEL findings [3,5]. **Statistical Analysis:** Non-paired Student's t-test was performed to determine the statistical significance (p<0.05).

## RESULTS:

There was a significant increase of TUNEL+ (apoptotic) chondrocytes in wild-type xiphoid as compared to the Bim<sup>-/-</sup>Bid<sup>-/-</sup> specimens (Figure 2A,2B). Additionally, wild-type specimens had considerably more caspase-3 positive cells than the Bim<sup>-/-</sup>Bid<sup>-/-</sup> knockout specimens (Figure 2C,2D). TUNEL was used to quantify the amount of apoptosis occurring in the injured specimens. At 48 hours, a statistically significant 54% decrease (p=0.027) was seen in the Bim/Bid double knockout mice as compared to the wild-type C57BL/6 mice (Figure 3). No notable differences were found between sham-operated wild-type and sham-operated Bim<sup>-/-</sup>Bid<sup>-/-</sup> groups.

## DISCUSSION:

Our findings indicate that a deletion of Bim/Bid can reduce but not prevent chondrocyte apoptosis in response to mechanical injury. This suggests that mitochondria plays an important role in apoptosis of chondrocytes after mechanical injury. Our results are consistent with the finding that the treatment of nitric oxide scavenger can reduce chondrocyte apoptosis after trauma injury, since Bim (mitochondrial intrinsic) pathway plays a role in mediating nitric oxide induced apoptosis [3]. Further studies are ongoing to determine whether this change is due to a delayed occurrence of apoptosis or long-term (>48 hours) prevention of chondrocyte apoptosis.

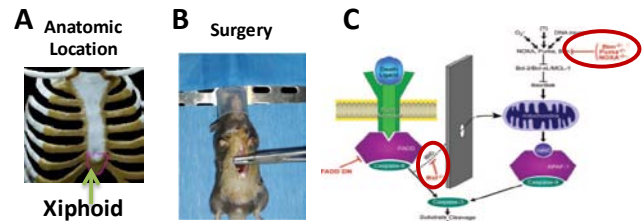


Figure 1. An illustration of *in vivo* xiphoid injured model and the roles of Bim/Bid in mitochondria-related apoptosis.

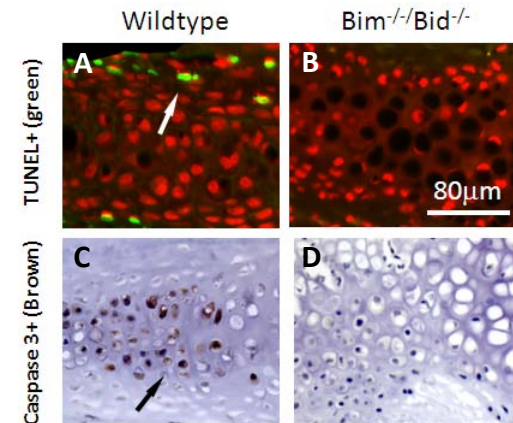


Figure 2. A reduction of TUNEL(A,B) and caspase 3 (C,D) positive chondrocytes was found in the Bim<sup>-/-</sup>Bid<sup>-/-</sup> mice as compared to the wild-type mice at 48 hours.

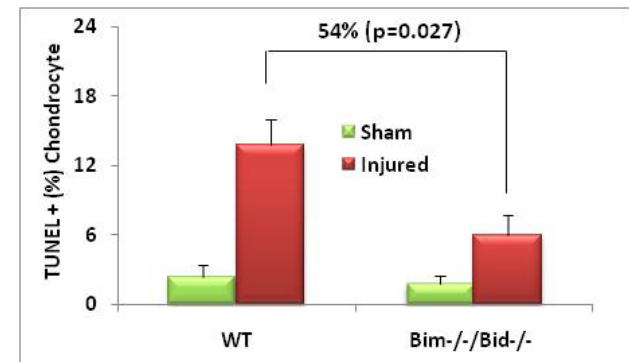


Figure 3. A significant decrease of apoptotic (TUNEL+) chondrocytes was found in injured Bim<sup>-/-</sup>Bid<sup>-/-</sup> xiphoid as compared to the wild-type group.

## References

1. Blanco+ Arth Rheum 1998;41:284-9.
2. Borrelli+ J Orthop Traum 2003;17:635-41.
3. Chang+ FASEB J 2007;21:708-19.
4. Chen+ J Orthop Res 1999;17:870-79.
5. Chen+ J. Orthop Res 2001;19:703-11.
6. D'Lima + Osteo Cart 2001 9:712-9.
7. Ewers+ J Orthop Res 2001;19:779-84.
8. Hashimoto+ Arth Rheum 1997;40:1749-55.
9. Loening + ABB 2000 381:205-12.
10. Martin+ JBJS 2009;91A:1890-7.
11. Repo and Finlay JBJS 1977;59A:1068-76.
12. Ward+ Arth Rheum 2008;58:744-53.

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