Role of HtrA1 in the Transition From Cartilage to Bone in Fracture Healing

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Background/Purpose: Endochondral bone formation is fundamental to the process of normal fracture healing. Central to the process of endochondral ossification is the production and destruction of chondrocytes along with their associated extracellular matrices (ECMs). The effects of mechanical stability have been studied extensively in bone remodeling but are lacking in the study of cartilage tissue turnover in fracture healing. Interestingly, a serine protease HtrA1 (high-temperature requirement protein A1) with cartilage ECM-degrading activity and mechanoresponsiveness has recently been identified. HtrA1 expression is restricted to the pericellular matrix (PCM) that immediately surrounds the chondrocyte. Taken together, these data strongly suggest that an understanding of the role of HtrA1 and the chondrocyte PCM in fracture healing is needed. We hypothesized that HtrA1 expression levels would vary temporally and spatially during the progression of fracture healing in mice. Additionally, we have asked whether these levels differ between rigid versus flexible internal femur fracture fixation situations. Such differences could implicate relationships between chondrocytes, HtrA1 expression, PCM turnover, and the inception of delayed fracture healing.

Methods: Fracture technique: Reproducible transverse femur fractures were generated via a traumatic three-point bending method with reproducible energy of injury in 8-week-old C57B/6 male mice. Quantitative real-time polymerase chain reaction (PCR): On sacrifice days 6, 8, and 10 the fractured limb callus was dissected free and callus tissue alone was placed in TRIzol reagent. The tissue was ground using a Polytron homogenizer and total RNA isolated using a commercially available kit. Potential DNA contamination was removed by RNase-free DNase treatment. Reverse transcription reaction was performed using 1ug of total RNA and random hexamer primers. Relative transcript levels were measured by quantitative PCR (qPCR) in an ABI PRISM 7000 FAST sequence detection system and were normalized to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA levels using commercially available primers and SYBR-Green master mix.

Results: HtrA1 mRNA levels were found to increase over the first 2 phases of fracture healing (inflammatory and cartilage formation) and peaked at day14 during the transition period from peak cartilage formation to primary bone formation/coupled resorption. qPCR revealed differences in gene expression events involved in chondrocyte pericellular matrix degradation between rigid versus flexible fractures. Flexible fixed fractures showed higher levels of mRNA for HtrA1 and discoid domain receptor 2 (DDR2) versus rigid fractures at day 7 of fracture healing. In addition, markers for early endochondral bone formation (vascular endothelial growth factor, collagen type II and collagen type X) were reduced in flexible femur fractures compared to rigid fractures.

Conclusion: In this study, we examined the effect of mechanical stability on chondrocyte behavior during the early stages of endochondral ossification in fractures. Our data validate a new methodology for studying the molecular mechanisms of mechanotransduction during fracture repair using relatively rigid or flexible fixation. In doing so, we are able to demonstrate an important role for the chondrocyte PCM in regulating the behavior of chondrocytes. These data provide insight into the potential role of both HtrA1 and DDR in regulating these early processes and suggest a potential role for pericellular chondrocyte signaling along the continuum of fracture healing from union to nonunion.

Alumni

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