Beta-catenin Signaling Plays a Disparate Role in Different Phases of Fracture Repair: Implications for Therapy to Improve Bone Healing

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Introduction: Delayed fracture healing causes substantial disability and usually requires additional surgical treatments. A pharmacologic management to improve fracture repair would substantially improve patient outcome. The signaling pathways that regulate bone healing are beginning to be unraveled, and they provide clues into pharmacologic management. The β-catenin signaling pathway has emerged as a key regulator in embryonic skeletogenesis. However, its role in fracture repair is unknown.

Materials and Methods: To investigate the role of β-catenin signaling during bone healing, we generated a stabilized tibia fracture in mice, and examined the β-catenin expression using Western analysis. We also performed LacZ staining in Tcf reporter mice to observe β-catenin mediated Tcf-dependent transcription during this regenerative process. To determine the role of β-catenin during fracture healing, we utilized a loss-of-function and gain-of-function approach. The repair process was observed in Catnb(tm2Kem) or Catnb(lox/ex3) mice, which conditionally express null or stabilized β-catenin alleles when subjected to an adenovirus expressing Cre-recombinase (Ad-Cre). To explore if β-catenin pathway in bone healing is regulated by Wnts, we treated mice with an adenovirus expressing Dickkopf-1 (Dkk-1, an antagonist of Wnt/β-catenin signaling). Furthermore, we generated α1(I)-Catnb(null) and α1(I)-Catnb(stab) mice that express osteoblast-specific β-catenin null or stabilized alleles, and observed fracture healing process in these mutants. To assess the ability of lithium to improve fracture repair, we also treated mice with LiCl either before or after the fracture, and compared the effectiveness of lithium on bone healing.

Results: Western analysis showed a significant upregulation of β-catenin during the entire healing process. Using LacZ staining, we observed that β-catenin mediated Tcf-dependent transcription was activated during fracture healing. Three days after the fracture, undifferentiated mesenchymal cells filled the fracture gap, and there was only small amount of LacZ staining. Nine days following the fracture, most of the callus was composed of cartilage. Cells surrounding the cartilage matrix showed strong staining. Chondrocytes also displayed staining signal. Osteoblasts along the trabeculae or periosteum displayed positive staining. Two weeks later, osteoblasts either lining the periosteum or along the islands of woven bone within the callus showed strong staining signal. However, the intensity of staining became fainter as osteoblasts matured to osteocytes. The callus was composed primarily of bone 3 weeks after the fracture, and cartilage was barely detected. LacZ staining was detected in osteoblasts but there was a very low level staining in the fully differentiated osteocytes. Five weeks later, the callus was composed of bone undergoing remodeling. LacZ staining was mainly detected in osteoblasts residing in periosteum.

To determine the role of β-catenin during bone healing, we observed the repair process in Catnb(tm2Kem) or Catnb(lox/ex3) mice, which conditionally express null or stabilized β-catenin alleles when subjected to Cre. Three weeks following the fracture, radiographic examination showed no evidence of bone healing in Catnb(tm2Kem) mice treated with Ad-Cre. To our surprise, in the Catnb(lox/ex3) mice whose β-catenin gene was conditionally stabilized after treatment of Ad-Cre, radiography also did not show evidence of bone bridging the fracture gap. Upon histological examination, fracture sites in Catnb(tm2Kem) mice mainly consisted of undifferentiated mesenchymal tissues, with no osteoblasts detected. Catnb(lox/ex3) mice showed a surprisingly similar histological appearance as appeared in Catnb(tm2Kem) mice, although less remaining cartilage was observed. These findings suggested a lack of both chondrogenic and osteogenic differentiation at the fracture sites in these two mice.

To investigate the contribution of Wnt ligands to β-catenin regulation in fracture repair, we treated mice with Ad-Dkk1. Three weeks later, all of the control animals showed complete healing, while in Dkk-1 treated mice, fractures failed to heal. There was a substantial reduction of both bone and cartilage volume in Dkk-1 treated mice, and the fracture site was mainly filled with large amount of undifferentiated tissues. These phenotypes were nearly identical to the results in mice expressing Cre-mediated β-catenin null alleles, suggesting a significant inhibition of bone healing by inactivation of Wnt/β-catenin pathway.

To determine the function of β-catenin in osteoblasts, we observed fracture healing in α1(I)-Catnb(null) and α1(I)-Catnb(stab) mice. At 1 week following the fracture, α1(I)-Catnb(null) mice exhibited a fracture callus mainly composed of cartilage matrix, while chondrogenesis was not evident in α1(I)-Catnb(stab) mice. Three weeks after the fracture, radiographic examination in α1(I)-Catnb(null) mice showed that the newly formed bone had not completely bridged the fracture gap, as compared to wild-type littermate. Also, a low bone density in α1(I)-Catnb(null) mice was observed, as compared to wild-type mice. Histological examination showed that the bone ends were not completely approximated in the α1(I)-Catnb(null) mice. Surprisingly, radiographic examination in α1(I)-Catnb(stab) mice showed an enhanced fracture healing, characterized by larger volume of regenerated bone tissues bridging the fracture gap as well as an increase in bone density, as compared to wild-type animals.

To assess the ability of lithium to alter fracture repair, we treated mice with lithium starting either 2 weeks prior to, or 4 days after the fracture. Mice in which the lithium treatment was started before operation had reduced bone in the fracture site, while mice in which the lithium treatment was started late displayed an enhanced fracture healing with relatively high radiopaque bone density. These data indicated that LiCl enhanced fracture healing, but only when utilized after the fracture when undifferentiated mesenchymal cells obtained osteoblast phenotype.

Discussion: In this study, we demonstrated that β-catenin signaling plays a crucial role during fracture healing. We observed that a precise regulation of β-catenin is important in the early phases of fracture healing to allow differentiation of mesenchymal cells into osteoblast and chondrocyte lineages. Based on our findings, β-catenin plays a disparate role in undifferentiated mesenchymal cells and in committed osteoblasts, and as such acts differently during different phases of fracture repair. These findings are important not only for understanding the role of β-catenin in different cell types, but also has a practical implication for therapy, as pre-fracture lithium treatment inhibits the repair process, however post-fracture lithium treatment enhanced bone healing. As such, lithium will enhance fracture healing, but only if started after cells have become committed to the osteoblast lineage.


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