

Key Transcriptional Differences Exist in Diabetic Fracture Healing

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Abstract

Background: Diabetes mellitus is a chronic disease associated with altered bone metabolism that leads to complications including non-union, malunion and delayed union of fractures. The mechanism(s) leading to impaired bone fracture healing remains to be elucidated. We hypothesize that bone formation and resorption are uncoupled in diabetic fracture healing, leading to impaired bone healing.

Methods: Bone marrow was isolated from femora of diabetic (db/db) and wild type control mice and cultured in RANKL and MCSF-enriched medium. After one week, assays were fixed for TRAP staining and analysis. Closed, bicortical femoral fractures were created in age- and gender-matched diabetic (db/db) and wild type mice. Radiography and mechanical testing were utilized to assess fracture healing. Femora and sera were harvested at different timepoints post fracture for histological, transcriptional and translational analysis.

The response to fracture injury in diabetic compared to control mice was analyzed using a Luminex multiplex assay to identify changes in circulating cytokines following femoral fracture. Quantitative PCR was performed to analyze differential gene expression of key osteoclastogenic, osteoblastogenic and adipogenic genes. Furthermore, microarray analysis was performed to identify genes that were differentially expressed in diabetic fracture callus compared to cells isolated from wild-type control.

Results: Bone marrow from diabetic mice formed significantly higher numbers of osteoclasts *in vitro* compared to wild type bone marrow (* $p < 0.05$). Osteoclasts formed from diabetic bone marrow were larger in size and had more nuclei per cell than wild type controls (* $p < 0.05$).

Diabetic mice showed delayed callus development on radiographic assessment *in vivo*. The diabetic fracture group were weaker in comparison to the wild-type control on mechanical testing (* $p < 0.05$). Histological analysis of fracture callus showed an increased deposition of adipocytes within the diabetic callus compared to wild-type callus (* $p < 0.05$).

Quantitative PCR identified a significant upregulation in transcription of genes contributing to osteoclastogenesis versus osteoblastogenesis (* $p < 0.01$). Notably, there was an upregulation of peroxisome proliferator-activated receptor gamma, which promotes adipocyte differentiation. Microarray analysis identified multiple further genes that were differentially expressed in diabetic fracture callus versus wild-type control.

Conclusions: Fracture healing is impaired in our murine model of diabetes. Our findings illustrate an upregulation of genes related to osteoclast and adipocyte function, with a concomitant reduction of osteoblastogenic gene expression in diabetic mice.

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