Metabolomic and Proteomic Characterization of the Human Fracture Microenvironment Milieu: The Effect of Patient Age

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INTRODUCTION:

Skeletal stem cells (SSCs) within the periosteum, endosteum, and bone marrow facilitate osseous regeneration after a fracture. In response to an injury, local signaling molecules are released which recruit SSCs to the damaged tissue and drive their proliferation and differentiation. The capability of SSCs to successfully repair bone is dependent on the proteins and metabolites found within the fracture microenvironment that guide the program of recruitment, proliferation, and differentiation. Aging is associated with impaired tissue regeneration including fracture repair. Previous work has demonstrated that chronic inflammation directly contributes to the elderly skeleton's reduced regenerative capacity. At the cellular level, inflammation leads to increased senescence within the SSC pool and reduces the number of cells available to repair a skeletal injury. The goal of this study was to determine how aging affects the levels of proteins and metabolites found within the fracture microenvironment.

METHODS:

The research study was reviewed and approved by an Institutional Review Board. All surgical procedures were performed by one of six board-certified orthopaedic surgeons with training in orthopaedic trauma surgery. Subjects aged 18 years old or older undergoing surgical intervention for a fracture were identified and invited to participate in the study from 7/1/2020 to 6/30/2021. Those diagnosed with rheumatological disease, metastatic cancer, or those who had previous chemotherapy treatment, previous radiation therapy, and/or previous chronic corticosteroid use were excluded. Fracture hematomas were collected intraoperatively with sterile curettes and syringes, processed, and analyzed by liquid chromatography-mass spectrometry. Protein and metabolite expression was compared for each fracture cohort.

RESULTS:

A total of 151 subjects were enrolled in the study with a total of 157 fracture hematomas submitted to the biorepository. From the biorepository, 7 subjects were selected for the young/hindfoot ankle cohort and 7 subjects were selected for the aged ankle/hindfoot cohort. The mean age and mean time from injury to the operating room for the young ankle/hindfoot cohort was 29.43 years old (range, 21-38) and 8.43 days (range, 6-11), respectively. The mean age and mean time from injury to the operating room for the aged ankle/hindfoot cohort was 59 years old (range, 45-70) and 8.57 days (range, 4-12), respectively. From the untargeted metabolomic analysis, 66 total metabolites were identified that were expressed at significantly different levels between hematomas collected from aged and young ankle/hindfoot fractures. Notably, creatine, 2-methylindoline, and acetyl-L-carnitine were generally expressed at higher levels in aged ankle/hindfoot fracture hematomas. No metabolites were identified from the global analysis that were expressed at higher levels in young ankle/hindfoot fracture hematomas. Proteomic analysis identified 34 proteins that were expressed at significantly different levels between the two cohorts.

DISCUSSION AND CONCLUSION:

Differences in the expression of metabolites and proteins within the fracture microenvironment are directly related to the age of the subject. Determining how these metabolites and proteins influence fracture healing and bone homeostasis is the goal of ongoing investigations.