

Murine Progeria Model Exhibits Delayed Fracture Healing and Elevated Local Immune Response

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INTRODUCTION: Fracture healing is well established to present with significant age-related delays and increased risk of nonunion. The NIH standard for age-related research in mice is 24 months of natural aging; a constraint that creates a substantial time and monetary burden to investigate mechanisms or therapies associated with age-related decline in bone healing. The *Zmpste24*^{-/-} (Z24^{-/-}) mouse model recapitulates the premature aged phenotypes of Hutchinson-Gilford progeria syndrome with genomic instability, epigenetic alterations, cellular senescence, stem cell exhaustion, and musculoskeletal deficiencies such as bone density loss, atypical skeletal geometry, sarcopenia, weight loss, osteoporosis, and osteoarthritis. Here we leverage the Z24^{-/-} mouse line to model delayed fracture healing in aged mice. Our primary hypothesis is that Z24^{-/-} mice will present with delayed fracture healing similar to naturally aged mice when compared to age-matched wild-type (WT) controls due to increased senescent cell burden and a systemic immune dysregulation.

METHODS: All procedures received IACUC approval and followed NIH guidelines for ethical treatment of animals. Age-matched WT (>18 months), Z24^{-/-} and WT controls (3-4 months) underwent right tibia fracture with intramedullary fixation. Mice were then sacrificed at specified time points post-fracture for analysis. Histomorphometry quantification and micro-CT analysis of the fracture callus was performed at 21- days post fracture and bone, cartilage, and fibrous tissue percentage was calculated. Whole blood samples obtained for all three groups via cardiac puncture and 25-50 μ L of blood from 3- and 14- days post fracture was used for immunophenotyping with a 32-color spectral flow panel that identifies 125 distinct populations, including major myeloid and lymphoid lineages. Z24^{-/-} and WT bone marrow was isolated from the contralateral tibia at 3- and 14- days post fracture and immunophenotyping analysis was performed with a 30-color spectral flow panel that identifies 76 distinct populations. Principle components analysis (PCA) was performed on the blood of all three groups for analysis of immunophenotyping data at 3- and 14- days post fracture. The same analyses were performed on the bone marrow of Z24^{-/-} and WT groups 3- days post fracture. Multiple group analyses were performed with ANOVA with Tukey's post-hoc test and two group analyses used unpaired t-test.

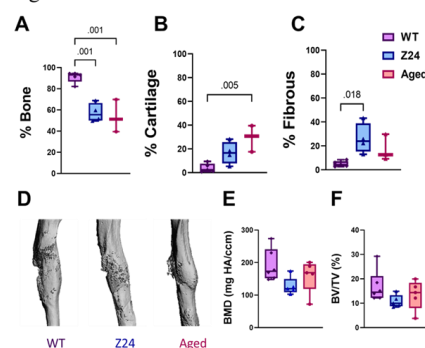
RESULTS: Histomorphometric quantification analyses at 21- days post fracture showed Z24^{-/-} (p=0.001) and aged (p=0.001) fracture calli had significantly lower bone volume compared to WT group (Figure 1A). Cartilage volume was significantly higher in aged mice (p=0.005) compared to WT mice (Figure 2B), while fibrous tissue volume was significantly higher in Z24^{-/-} mice (p=0.018) compared to WT mice (Figure 2C). Representative micro-CT images of fracture callus showed no significant changes in bone mineral density and ratio of bone volume to total callus volume between all three groups (Figure 1D-F). Immunophenotyping analysis of blood at 3- and 14- days post-fracture saw few differences between WT and Z24^{-/-} mice and few similarities between aged and Z24^{-/-} mice, whereas WT and aged displayed unique immunophenotypes (Figure 2A-B). Further blood PCA analyses confirmed many similarities between WT and Z24^{-/-} mice with the aged group displaying unique immunophenotype (Figure 2C) while establishing sex immunophenotype differences (Figure 2D). Flow immunophenotyping bone marrow analysis established many differences between WT and Z24^{-/-} groups at 3- days post fracture, but few differences at 21- days post fracture (Figure 3A). Bone marrow PCA analyses confirmed that WT and Z24^{-/-} showed few similarities in immunophenotype (Figure 3B). A significant decrease in B lineage leukocytes in Z24^{-/-} versus WT mice (p=0.0004) was observed, whereas WT mice showed elevated myeloid lineage (p=0.0007), CD80 monocytes (p=0.0161), C12FDG+ monocytes (p=0.0026), and C12FDG+ NK cells (p=0.0029) compared to Z24^{-/-} mice (Figure 3C-E).

DISCUSSION: These results suggest that although the Z24^{-/-} model recapitulates the delayed fracture healing commonly associated with aging, this murine model may not be related to systemic immune dysregulation. Immunophenotyping data show that while WT and aged mice displayed expected differences, Z24^{-/-} mice were more similar to their WT counterparts than to aged WT mice, indicating that Z24^{-/-} mice do not display an aged systemic immune response. This finding, combined with dysregulated immune response in Z24^{-/-} bone marrow and contralateral lymph nodes (data not shown) suggest that Z24^{-/-} mice delayed fracture healing is a local rather than a systemic immune response. Further data is required to elucidate the relationship between Z24^{-/-} delayed fracture healing and specific immune response pathways.

SIGNIFICANCE/CLINICAL RELEVANCE: Currently, there is a paucity of feasible delayed fracture healing models. This study further details the potential mechanisms contributing to delayed fracture healing in Z24^{-/-} mice, establishing its value as a model. As we reveal more about this model, it can be leveraged to reduce time and monetary burden for studies regarding novel therapeutic strategies for delayed fracture healing.

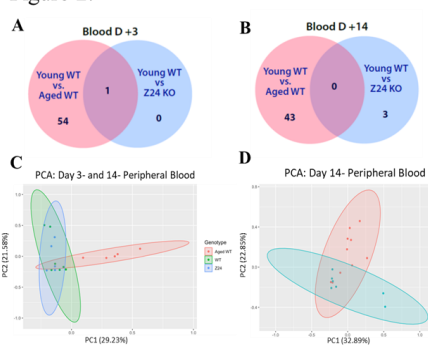
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Figure 1.



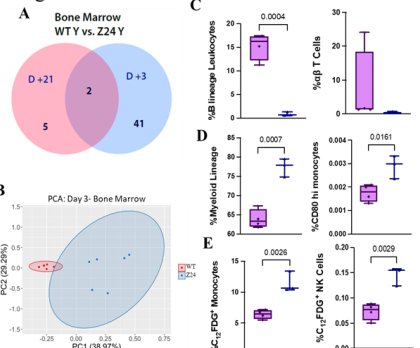
(A) 21- day post fracture histomorphometry quantification of bone%, (B) cartilage%, (C) and fibrous tissue% in WT, Z24^{-/-} and aged WT mice. (D) Representative micro-CT images of fracture callus bone architecture. (E) Bone mineral density and (F) ratio of bone volume to total callus volume.

Figure 2.



(A) Spectral flow immunophenotyping differences between WT, Z24^{-/-}, and aged WT blood at 3- days and (B) 14- days post fracture. (C) Peripheral blood PCA analyses of WT, Z24^{-/-}, and aged WT mice at 3- and 14- days post fracture and (D) sex differences at 14- days post fracture.

Figure 3.



(A) Spectral flow immunophenotyping differences between WT and Z24^{-/-} bone marrow at 3- and 21- days post fracture. (B) PCA analyses of WT and Z24^{-/-} bone marrow 3- days post fracture. (C) Comparison between WT and Z24^{-/-} expression of B-lineage leukocytes and α B T cells, (D) myeloid lineage and CD80 monocytes, (E) C12FDG+ monocytes and C12FDG+ NK cells.