



Deficient Geriatric Fracture Healing is Associated with Alterations in Immune Cell Function and Cell Cycle

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Introduction

Fragility fractures lead to significant patient morbidity, mortality, and cost to both the individual and society.^{1,2} Geriatric patients often experience a protracted healing course and are at higher risk for poor outcomes. Discriminating underlying differences in local gene expression at the fracture site can be used to understand the mechanistic dysfunctions in geriatric healing. The objective of this study was to compare fracture healing gene expression profiles of young and geriatric mice in a traumatic long bone fracture model.

Methods

Five month-old (mo) mature but young mice and 25 mo geriatric mice underwent bilateral, closed, traumatic 3-point bend tibial diaphyseal fractures with intramedullary pin fixation. Five mo “young adult” mice and 25 mo “geriatric” mice (corresponding to human age of 70-85)³ were obtained from the National Institute of Aging (NIA) C57BL/6 colonies in which geriatric mice have been shown to have delayed and decreased fracture healing^[4]. Tibiae were harvested at 0, 5, 10, and 20 days post fracture (DPF). RNA was harvested from homogenized callus. Global gene analysis was performed using the Affymetrix MoGene v1 r4 array. Significance was determined by a t-test using permutation based false-discovery rate (FDR) and $p < 0.05$ with MeV software. Positive genes were uploaded into DAVID bioinformatics for gene set enrichment analysis (GSEA) and uploaded to Cell Type

Enrichment Analysis for Microarray Data (CTen) for determination of cell populations present throughout fracture healing.

Results

Time from fracture (DPF) was the strongest determinant of global expression profile change with age being a modifying factor. CTen analysis showed changes in gene expression profiles consistent with increases in stem cells and osteoblasts in both young and old mice between 0, 5 and 10 DPF and a reduction by 20 DPF. In contrast, a large relative decrease in macrophages was predicted in young mice from 5 to 10 DPF but not in geriatric mice. Additionally, geriatric mice exhibited an increase in CD8+ T-cell expression not seen in young mice (10-20 DPF). GSEA DAVID analysis provided ranked differences in numerous pathways (Table 1); most strikingly, pathways that are different between aged and young healing are related to the immune system and cell cycle. As an example, cell cycle related genes are highly expressed early (0, 5, and 10 DPF) in young mice but later (20 DPF) in geriatric mice (Figure 1).

Discussion

As expected, time post-fracture was more predictive of changes in gene expression than chronological age. However, differences in the timing and duration of the immune response and the regulation of the cell cycle were present. These differences may be partially responsible for the observed deficiencies in aged fracture

Table 1. DAVID gene set enrichment analysis showing the top pathways that differ between young and old mice

DPF	Pathways
0	1. Antigen processing and presentation; 2. Cell adhesion molecules (CAMs); 3. Graft-versus-host disease; 4. Allograft rejection; 5. Type 1 diabetes mellitus
5	1. Glycerolipid metabolism; 2. Cell cycle; 3. Prostate cancer; 4. Focal adhesion; 5. Biosynthesis of unsaturated fatty acid
10	1. Regulation of actin cytoskeleton; 2. Pathways in cancer; 3. ECM-receptor interaction; 4. Focal Adhesion; 5. Chronic myeloid leukemia
20	1. Leukocyte transendothelial migration; 2. Chemokine signaling pathway; 3. Focal Adhesion; 4. Natural Killer Cell Mediated cytotoxicity; 5. Fc epsilon RI signaling pathway

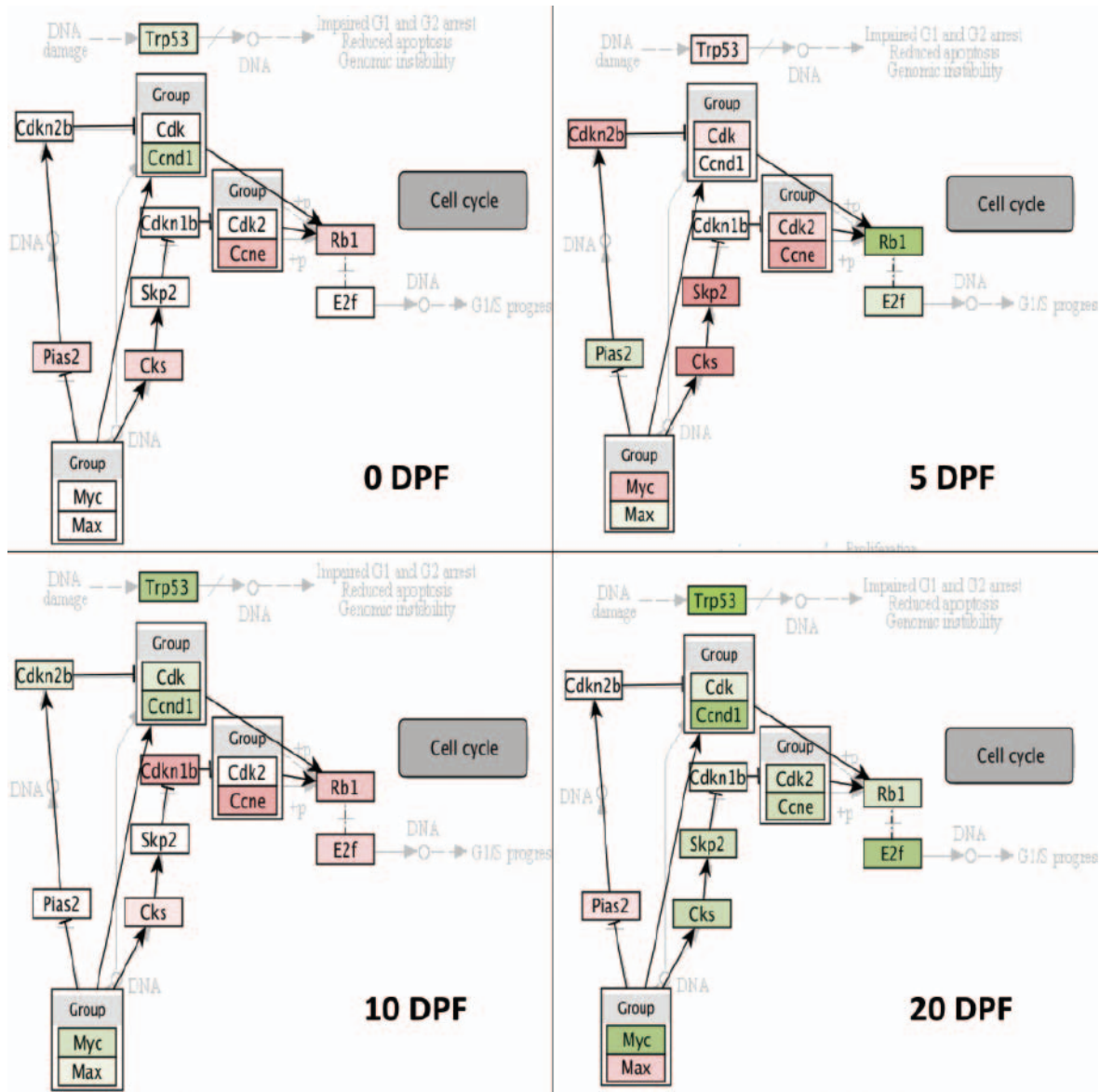


Figure 1. Expression of genes involved in the cell cycle. Diagrammatic representation of cell cycle genes that are changed in association with fracture healing of young and geriatric mice. Red coloring indicates higher expression in young mice, whereas green coloring indicates higher expression in geriatric mice. Note the generally enhancing levels of cell cycle genes at 0, 5, and 10 DPF in young mice, while at 20 DPF, geriatric mice show increases in cell cycle-associated genes relative to young mice.

healing and potentially be targets for improving fracture healing in the future.

Significance

Understanding global genomic expression and cell population patterns in murine geriatric fracture patterns can lend insight into the fundamental biology of altered fracture healing in aged animals. This knowledge can be used for further investigation and manipulation of rationally selected deficits in aged fracture healing.

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