Transient Expansion and Myofibroblast Conversion of Marrow Adipogenic Lineage Precursors (MALPs) Mediate Bone Marrow Repair after Radiation

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Disclosures

None

Introduction

Radiotherapy treats malignant tumors effectively but also damages surrounding tissues, such as bone marrow. It is well known that radiation causes a collapse of bone marrow cells and elimination of microvasculature. At a less severe level, radiation damage can be reversed, indicating that bone marrow has a regenerative ability. However, the mechanism governing such recovery is still largely unknown. Apart from hematopoietic cells and endothelial cells, mesenchymal lineage cells are also a major component of bone marrow providing supportive microenvironment for hematopoiesis and angiogenesis. Using single cell RNA-sequencing (scRNA-seq) technique, we recently discovered a novel subpopulation of mesenchymal cells that express most adipogenic markers but with no lipid accumulation and named them marrow adipogenic lineage precursors (MALPs)[1]. In this study, we utilized a modest focal radiation dosage to generate a bone marrow repair model and investigate the underlying mechanism.

Methods

Animals

All animal work was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. *Col2-Cre Rosa-Tomato (Col2/Td)* mice and *Adipoq-Cre Rosa-Tomato (Adipoq/Td)* mice were generated. For focal radiation, mouse right femur received a clinically relevant radiation dose of 5 Gy using small animal radiation research platform (SARRP).

scRNA-seq analysis

Sorted Td^+ cells from the endosteal bone marrow of 1-month-old *Col2/Td* male mice with no radiation (NR, 2 batches, n = 5) or at 3 days after radiation (R, 1 batch, n = 3) were subjected to library construction and sequencing. Unsupervised clustering was conducted by UMAP to generate cell clusters of the overall cell populations. Pseudotime trajectory analysis was conducted using Monocle 3.

Whole mount immunofluorescence

Bones were processed for 50 μm-thick whole mount cryosections and stained with indicated antibodies.

Statistics

All analyses were conducted using t-tests or one-way ANOVA with Tukey post test.

Results

5 Gy focal radiation to femurs of 1-month-old mice caused acute damage on bone marrow cellularity and vasculature at day 3, which was recovered at day 14. Using *Col2-Cre* that labels the entire mesenchymal lineage cells in bone[2,3], we unexpectedly observed a 5.5-fold increase of bone marrow Td^+ cells at day 3 after radiation, which later gradually disappeared. ScRNA-seq on Td^+ cells sorted from NR or R bones at day 3 generated the same clustering pattern consisting of early mesenchymal progenitors (EMPs), late mesenchymal progenitors (LMPs), lineage committed progenitors (LCPs), osteoblasts, osteocytes and MALPs and the same pseudotime trajectory pattern (Fig.1 A, B). After integration of 2 datasets, we found that the mesenchymal progenitor pool including EMPs and LMPs was drastically shrunk while MALPs were greatly expanded after radiation (Fig.1 C). Interestingly, cell cycle analysis revealed that MALPs, a non-proliferative cell type, became highly proliferative after radiation (Fig1.D). Analyzing differentially expressed genes (DEGs) found that radiation upregulates many myofibroblast (*Acta2, Tagln, Myl9* etc) and extracellular matrix (*Col9a1, Col10a1, Col1a1* etc) genes in MALPs, indicating a myofibroblast transformation. Using *Adipoq-Cre* to label MALPs *in vivo*, we observed that radiation significantly increased the number of MALPs and its proliferation (EdU^+Td^+ cells) at day 3 post radiation (Fig.2). qRT-PCR of sorted Td^+ cells from *Adipoq/Td* mice confirmed that myofibroblast markers are indeed increased

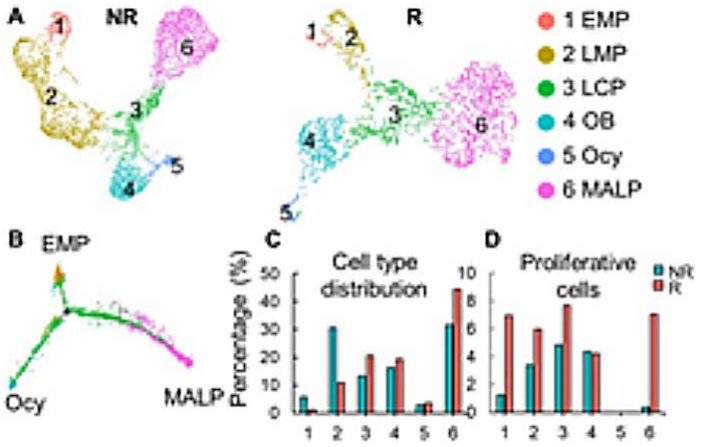

Figure 1. ScRNA- seq analysis predicts MALPs expansion and cell cycle entry after radiation. **(A)** The UMAP plot of Td$^+$ mesenchymal lineage cells isolated from 1 month-old Col2/Td mice with no radiation (NR) or at 3 days after radiation (R). **(B)** Monocle trajectory plot from R dataset. **(C)** The distribution of cells from NR and R datasets in each cluster. **(D)** The percentage of proliferative cells (S/G2/M phase) among each cluster in NR or R conditions was quantified.

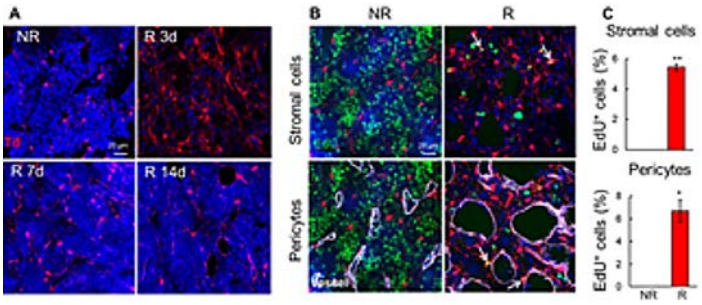

Figure 2. Radiation transiently expands MALP pool via stimulating its proliferation. **(A)** Fluorescent images of Td$^+$ cells in the endosteal bone marrow of 1-month-old *Adipoq/Td* femur before and after focal radiation. **(B)** Fluorescent images of EdU incorporation in bone marrow cells of *Adipoq/Td* femurs at 3 days post radiation. Arrows point to EdU$^+$Td$^+$ cells. **(C)** Quantification of EdU$^+$ cells in both stromal and pericytes from *Adipoq/Td* mice.

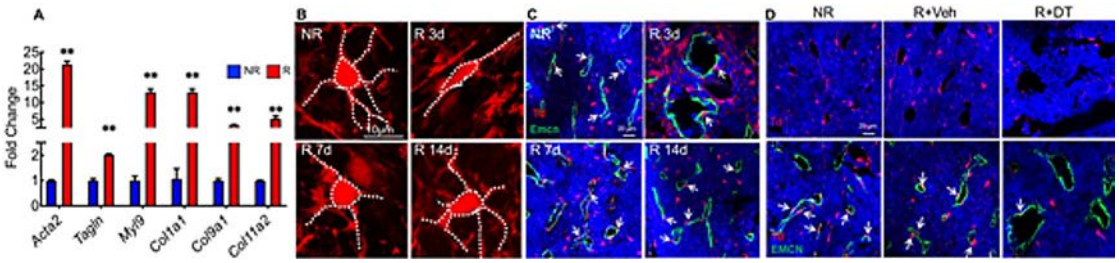

Figure 3. MALPs acquire myofibroblastic features and are indispensable for bone marrow repair after radiation. (A) qRT-PCR of sorted bone marrow Td$^+$ cells from *Adipoq/Td* mice. **(B)** Cell processes in 3D image of MALPs. **(C)** Fluorescent images of vessel and Td$^+$ pericytes on vessel post radiation. **(D)** Fluorescent images of vessel and Td$^+$ pericytes on vessel after radiation and DT treatment.

(Fig.3 A). MALP is composed of a cell body with several cell processes. Strikingly, radiation diminished the number of its cell processes and changed its shape to more spindle-like (Fig.4 B). Many MALPs exist as pericytes. Td$^+$ pericytes in *Adipoq/Td* mice decreased by 76% along with vessel dilation at day 3 post radiation but returned to normal numbers with relatively normal vessel structure at day 14 (Fig.3 C). Ablation of MALPs in *Adipoq/Td/DTR* mice after radiation almost completely abolished the recovery of bone marrow cellularity (decreased by 85%) and vessel structure at day 14 (Fig3. D).

Discussion

In the adipogenic differentiation route of bone marrow mesenchymal stem cells, MALPs are situated after mesenchymal progenitors and before lipid-laden adipocytes (LiLAs). They normally do not proliferate. Our study on radiation effects on bone discovered that MALPs start to proliferate and acquire myofibroblastic features after radiation, which are essential for subsequent bone marrow repair. Our data revealed the plasticity of MALPs and shed light on seeking new target for alleviating radiation damage on bone.

Significance

MALPs play a crucial role in bone marrow regeneration after radiation injury.

References

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