Identification of a Novel Adipose Lineage Cell Population that Regulates Bone Marrow Environment

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Introduction

Bone marrow adipocytes are conventionally viewed as large cells containing unilocular lipid droplets. Originally considered as space fillers, they are now thought to be a negative regulator of osteogenesis because both adipocytes and osteoblasts are derived from bone marrow mesenchymal stem cells (MSCs). We recently applied single cell RNA-sequencing (scRNA-seq) on sorted bone marrow mesenchymal lineage cells from 1-mo-old mice that has very few marrow adipocytes. Unexpectedly, we identified a large mesenchymal subpopulation that expresses many mature adipocyte markers (*Pparg, Cebpa, Adipoq, Apoe*, and *Lpl*) but not liqid droplet-associated genes (*Perilipin* and *Fabp4*). Here, we constructed mature adipocyte-specific *Adipoq-Cre(ER) Rosa-tdTomato (Adipoq(ER)/Td)* mice to validate this novel cell population and study their actions in bone.

Methods

Animals

All animal work performed was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. *Col2/Td*, *Adipoq/Td, AdipoqER/Td* mice were obtained by crossing *Rosa-tdTomato* mice with *Col2-Cre*, *Adipoq-Cre, Adipoq-CreER* mice, respectively. *Adipoq/Td/DTR* mice were generated by breeding *Adipoq/Td* mice with *Rosa-DTR* mice. *AdipoqER/Td* mice received Tamoxifen (Tam) injections (75 mg/kg/day) at P6 and P7 and euthanized at 1 mo of age. *Adipoq/Td/DTR* mice received vehicle or diphtheria toxin (DT, 50 μg/kg) every other day for 2 wk. For focal radiation, mouse right femur received a clinically relevant radiation dose of 5 Gy using small animal radiation research platform (SARRP).

Immunofluorescence

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

Transplantation

Freshly FACS-sorted Td^+ cells ($5x10^4$/transplant) were mixed with Gelfoam and placed under the kidney capsule of 2-mo-old *C57Bl/6* mice. 4 wk later, mice received calcein injection (15 mg/kg) 1 day before harvesting grafts.

Statistics

All analyses were conducted using t-tests.

Results

Td labeled all $Perilipin^+$ adipocytes, many $CD45^-$ stromal cells, and pericytes, but not osteoblasts, osteocytes, and chondrocytes in 1-mo-old *Adipoq/Td* mice (Fig.1A). The majority of Td^+ cells (99.8%) did not harbor lipid droplets

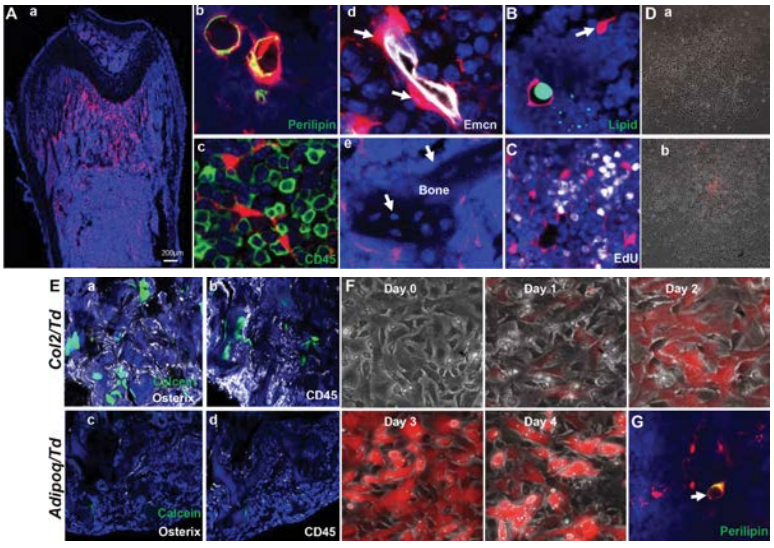

Figure 1. Mouse bone marrow contains abundant non-lipid-laden adipocytes. **(A)** In a 1-mo-old *Adipoq/Td* femur (a), Td labels $Perilipin^+$ adipocytes (b), $CD45^-$ stromal cells (c), pericytes (d), but not osteoblasts and osteocytes (e). **(B)** BODIPY lipid staining shows a Td^+ stromal cell (arrow) with no lipid. **(C)** Td^+ cells do not incorporate EdU. **(D)** all CFU-F colonies are made of Td^- cells (a) while some Td^+ cells do attach to the dish (b). **(E)** Td^+ cells from *Col2/Td* mice, but not *Adipoq- Td* mice, form bone-like structure. **(F)** In vitro adipogenic differentiation assay of Td^- mesenchymal progenitors from *Adipoq/Td* mice. The same area was imaged daily. **(G)** Bone marrow $Perilipin^+$ adipocytes are derived from non-lipid-laden Td^+ adipocytes in 1-mo-old *AdipoqER/Td* mice (Tam injections at P6, 7 when no $Perilipin^+$ cells can be detected in the bone marrow).

and none of them incorporated EdU (Fig.1B,C). Td^+ cells constituted ~18% of $CD45^-Ter119^-$ bone marrow cells. After isolation, they attached to the culture dish but did not form CFU-F colonies (Fig. 1D). While Td^+ cells from *Col2/Td* mice (in which Td labels mesenchymal progenitors) formed bony structure after transplantation, freshly sorted Td^+ cells from *Adipoq/Td* mice did not (Fig.1E), indicating that they are not mesenchymal progenitors. Upon adipogenic differentiation, Td^- mesenchymal progenitors from *Adipoq/Td* mice first became Td^+ cells with no lipid droplets and then evolved into Td^+ cells with lipid (Fig. 1F). Similar cell culture results were also obtained with *AdipoqER/Td* mice. Fate mapping in postnatal *AdipoqER/Td* mice confirmed that $Perilipin^+$ lipid-laden adipocytes are derived from non-lipid-laden Td^+ adipocytes (Fig. 1G). In the bone marrow of young *Adipoq/Td* mice, all pericytes identified by PDGFRβ or Laminin staining in a peri-capillary location were Td^+ (Fig. 2A). Strikingly, using whole mount sectioning and confocal scanning, we found that bone marrow Td^+ stromal cells and pericytes form a 3D network made of cell processes to communicate amongst themselves and other components of bone, including vessel walls (Fig 2B). Ablating those cells in 1-mo-old *Adipoq/Td/DTR* mice after 2 wk of DT injections disrupted bone marrow vasculature and caused drastic *de novo* bone formation in the diaphyseal bone marrow (Fig. 3), suggesting that those cells function in maintaining vessel integrity and inhibiting osteogenesis. Focal radiation on long bones rapidly expanded the non-lipid-laden Td^+ cells at d3, accompanied with vessel dilation and a loss of Td^+ pericytes (Fig. 4). By d7, both vessel structure and Td^+ pericyte density returned to relatively normal levels. These data implied a role of Td^+ adipocytes in the repair and stabilization of marrow vessels after radiation injury.

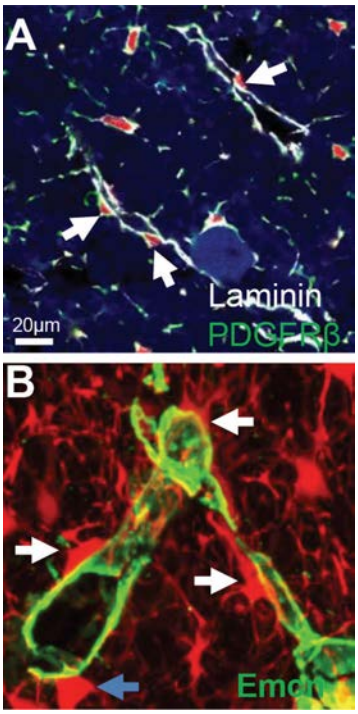

Figure 2. Non-lipid-laden Td^+ cells are stromal cells and pericytes forming a 3D network inside the bone marrow. **(A)** All $PDGFR\beta^+$ and $Laminin^+$ cells with a pericyte morphology are Td^+ (arrows). **(B)** Td^+ stromal and pericytes are morphologically similar with many cell processes.

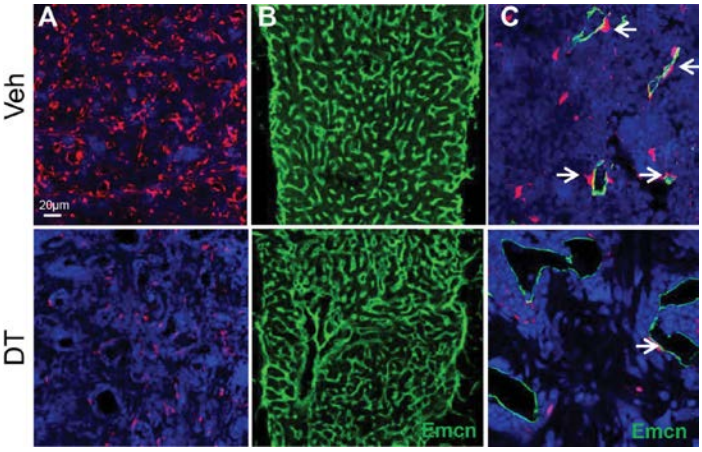

Figure 3. Ablation of adipocytes reveals their roles in maintaining vasculature and bone. **(A)** Bone marrow Td^+ cells in *Adipoq/Td/DTR* mice were ablated by DT. **(B)** DT altered bone marrow vessel structure. **(C)** High magnified image showed that vessels were dilated coinciding with a depletion of Td^+ pericytes **(D)** 3D μCT images show drastic *de novo* bone formation in femoral midshaft after DT injections.

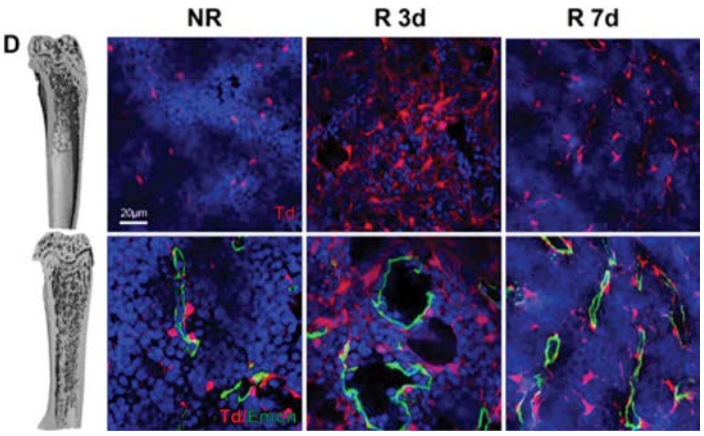

Figure 4. Adipocytes are required for the recovery of bone marrow vasculature after radiation injury. Fluorescent images of total Td^+ cells (top) and pericytic Td^+ cells (bottom) in the bone marrow of 1-mo-old *Adipoq/Td* femurs before (NR) and after (R) focal radiation (3 and 7 days).

Discussion

Our study demonstrate that bone marrow contains a large number of non-proliferative, mature adipocytes with no significant lipid stores. Those cells represent a stable transitional cell type situated after mesenchymal progenitors and before classic lipid-laden adipocytes along the adipogenic differentiate route. They are morphologically and functionally distinct from traditional adipocytes. Existing as stromal cells or pericytes, they possess numerous cell processes to form a vast 3D network structure in bone marrow. Our scRNA-seq data suggest that they express many secretory factors, including angiogenic factors. Most likely through secreting these factors into marrow environment, they play pivotal roles in maintaining marrow vasculature, suppressing osteogenic differentiation of mesenchymal progenitors, and participating into vessel repair after radiation injury. Therefore, we name them marrow environment regulating adipose cells (MERAs).

Significance

We discovered a novel type of adipose lineage cell population that regulates bone marrow environment.