Intramedullary thrombopoiesis and β-1,4-Galactosyltransferase 1 Is Required for maintenance of the vascular integrity in the bone marrow.

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Glycosyltransferases catalyze the transfer of sugar moieties from activated donor to acceptor molecules that determines the biosynthesis of glycans. β1,4-galactosyltransferase 1 (β4GalT1) transfers galactose to GlcNAc with a β1,4 linkage to produce a disaccharide called type-2 lactosamminyl glycan or LacNAc. Recent findings show that β4GalT1 deficiency increase the numbers of mature megakaryocytes in the bone marrow but due to increase in β1 Integrin signaling MK thrombopoiesis is severely impaired leading to thrombocytopenia.

Here, we investigated the role of β4galt1 in bone marrow recovery after chemotherapeutic 5-Fluoroacil (5FU) injury. β4galt1$^{-/-}$ mice and wild type mice were treated with FU and bone marrow was collected at day 0, 5 and 10 after treatment for bone marrow morphology and endothelial compartment analysis.
β4galt1$^{-/-}$ bone marrows had increased arterial and decreased sinusoidal compartments at steady state compared to controls, as determined by phenotypic flow cytometry sorting of endothelial cells and immunofluorescence analysis.

As expected, 5-FU administration led to reduced cellularity and formation of apparently cell-free cavities in the center of the femur shaft of wild type and β4galt1$^{-/-}$ mice. Further high-resolution 3D reconstruction of the cavity compartment revealed that while megakaryocytes localize preferentially at the edges of the cavity in control and β4galt1$^{-/-}$ bone marrows. In control bone marrows megakaryocytes release proplatelets (megakaryocyte cytoplasmic extensions) and platelets into the cavity, whereas by contrast, the cavity remained platelet-free in β4galt1$^{-/-}$ bone marrows, despite increased numbers of fully matured megakaryocytes at the edges of the cavity. Interestingly, proplatelets are released into the cavity post injury thereby distributing glycosaminoglycans and platelet content, suggesting that platelets may model the cavity to promote vascular growth. At day 10 post-treatment, control bone marrow vasculature compartment was comparable to control bone marrows, by contrast the β4galt1$^{-/-}$ vascular compartment was severely impaired, suggesting that proplatelet release is necessary for vascular recovery post injury. To investigate the role of proplatelets and platelets in the rebuilding process of the bone marrow post injury, platelets were depleted using anti-GPIbα antibodies. In control mice, injection of the anti-platelet antibodies depleted the cavity of platelet material leading to severely delay in the recovery of the bone marrow vascular compartment.

Together these results show that intra-medullar thrombopoiesis and platelet release and the presence of β4galt1 in megakaryocytes is required for maintenance and recovery of the vascular bone marrow compartment.