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Abstract Title: Loss of mannose-6-phosphate receptor in mice alters hematopoietic stem and progenitor cell population and causes abnormal megakaryocyte maturation

Blood platelets are produced in the bone marrow by megakaryocytes (MKs) in unique processes that require polyploidization, extensive membrane rearrangements, and the formation of secretory granules, including α-granules, dense granules, and lysosomes. Lysosomal enzymes are targeted to the lysosome via the mannose 6-phosphate receptor (M6PR) pathway, deficiency of which has been associated with thrombocytopenia. Lysosomal enzymes bearing phosphomannosyl residues specifically bind M6PRs in the trans-Golgi network (TGN) and the resulting M6PR-enzyme complex is transported to an acidic late endosomal/pre-lysosomal compartment, where the low pH mediates the dissociation of the complex. Lysosomal enzymes are then further transported to established lysosomes, while M6PRs recycle back to the TGN to repeat the process. To determine the role of M6PR-specific targeting of lysosomal enzymes in platelet production and function, platelet and megakaryocyte parameters were investigated in M6pr–/– mice lacking the 46-kDa M6PR, the physiological role of which is unclear. M6pr–/– mice had significantly increased platelet counts with an abnormally elevated number of circulating proplatelets, appearing as thin and elongated platelet strands, compared to single discoid platelets in controls. Consistent with the increase in circulating platelets and their abnormal morphology, transmission electron microscopy revealed the presence of an abnormal demarcation membrane system (DMS) in M6pr–/– bone marrow MKs with poorly defined platelet territories. However, M6pr–/– mice had normal bone marrow MK counts. Flow cytometry analysis of bone marrow hematopoietic stem and progenitor cells revealed a reduction in the MK-erythroid progenitors population in M6pr–/– mice. Bone marrow M6pr–/– MKs in culture showed multilobulated nuclei surrounding a highly disorganized Golgi apparatus. The results indicate that M6PR-specific targeting of lysosomal enzymes plays a critical role in the formation of the DMS in MKs, thereby regulating platelet production.