Submission #4

Untitled

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Abstract Title: M6PR-specific targeting of lysosomal enzymes regulates platelet production and function in mice

Platelets are small, anucleate blood cells produced within the bone marrow (BM) by hematopoietic precursors known as megakaryocytes (MKs) through a series of intricate 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, and deficiencies in this trafficking have been associated with thrombocytopenia. Lysosomal enzymes bearing phosphomannosyl residues specifically bind M6PRs in the trans-Golgi network (TGN) and the resulting M6PR-enzyme complex translocates to the acidic late-endosomal compartment, where a low intraluminal pH mediates the dissociation of the complex. Whereas the M6PR is recycled back to the golgi apparatus following this separation, the trafficked lysosomal enzyme is further transported to established lysosomes. To determine the role of M6PR-specific targeting of lysosomal enzymes in platelet production and function, platelet and MK parameters were investigated in M6pr−/− mice lacking the 46-kDa M6PR, the physiological role of which is unclear. M6pr−/− mice had a severe bleeding diathesis, based on the tail bleeding time assay. However, M6pr−/− platelets normally form occlusive thrombi in vivo in an arterial FeCl3 injury model and adhered to type IA collagen with significantly greater propensity than control platelets in in vitro flow experiments. 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. M6pr−/− platelets expressed major glycoproteins on their surface and von Willebrand factor and fibrinogen in their α-granules. Transmission electron microscopy (TEM) revealed the presence of abnormal membrane tubulations, elongated and electron-dense granules, and large vacuole-like structures within resting M6pr−/− platelets. Moreover, and consistent with their increase in circulating platelets and their abnormal morphology, electron micrographs further uncovered significantly altered demarcation membrane system (DMS) development in M6pr−/− bone marrow MKs with poorly defined platelet territories. Taken together, the results suggest that the M6PR-specific targeting of lysosomal enzymes plays a critical role in platelet function and in the formation of the MK DMS, thereby regulating platelet production and platelet-mediated hemostasis.