Understanding Fibrinolysis in Sickle Cell Disease: Characterization of in vitro Blood Clot Resolution by Monocytes

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Sickle Cell Disease (SCD) is a hereditary blood disorder. People with SCD experience pain crises, hypercoagulation, and strokes, and suffer dramatically shortened lifespans. SCD causes individuals to produce abnormal hemoglobin that distorts red blood cells (RBCs) into a sickle shape when deoxygenated, causing blockage of blood vessels and damage to tissue that causes an increase in abnormal blood clots. Blood clots are formed from fibrin meshwork that then captures RBCs to prevent blood loss. Cathepsins are powerful proteases that are upregulated in SCD and have recently been shown to have fibrin(ogen)olytic properties. To uncover mechanisms for aberrant clot resolution in SCD, this project examines fibrin degradation by inflammatory cells, specifically monocytes, known to be upregulated due to chronic inflammation in SCD. To confirm monocytes are fibrinolytic, fibrinogen cathepsin zymography was run on THP1 monocyte cell lysates to quantify amounts of active cathepsins in monocytes. From this, monocytes were seeded onto fibrin gels polymerized in a 12-well plate to visualize in vitro degradation by monocyte produced enzymes, to determine the role of cathepsins and monocytes in resolving fibrin-based gels as an analogy of the clots. Gels were imaged at 0 and 24 hours to visualize fibrin degradation. After 24 hours gels showed visible degradation and samples were collected to quantify protease activity. Reducing SDS-PAGE was run to assess degradation of fibrin at the molecular level at the alpha, beta, and gamma polypeptide chains of fibrin, and the resulting gel confirmed fibrin degradation by proteases produced by monocytes. These results provide evidence for monocyte and cathepsin-mediated fibrin degradation indicative of cathepsin involvement in fibrinolysis in SCD.

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