BCR 70

Development of a plasminogen activator 1 (PAI-1) variant to modulate bleeding

Haynes, Laura; Huttinger, Zachary; Ginsburg, David

Submission Group

Biomedical/Coagulation Research

Abstract

Objective: PAI-1 is a serine protease inhibitor (SERPIN) whose function in vivo is to downregulate fibrinolysis by inhibiting urokinase- and tissue-type plasminogen activators (uPA and tPA). The goal of this research is to use PAI-1 as a prototypical SERPIN scaffold from which to develop “designer” PAI-1 variants with altered specificity and inhibitory kinetics. Methods: PAI-1 variants of interest were identified using a molecular evolution approach in which traditional phage-display technology was coupled with next generation high throughput DNA sequencing. Filamentous phage displaying PAI-1 fused to the p3 coat protein were randomly mutagenized through error prone PCR, reacted with uPA, and selected with an anti-uPA polyclonal antibody in two sets of complimentary experiments to (1) determine the stable half-life of PAI-1 or (2) the rate at which PAI-1 inhibits uPA. At selected time points in each of the described experiments, the PAI-1 encoding portion of the phage genome was analyzed by next-generation sequencing. Evaluation of 7-10 million sequencing reads per time point facilitated massively parallel kinetic analyses to determine the effect of amino acid substitutions at every residue in PAI-1 simultaneously with respect to both half-life and rate of uPA inhibition. Summary of Results: This analysis generated data for 74% of all possible single amino acid substitutions, identifying 492 mutations that extended the functional half-life of PAI-1, as well as 1509 destabilizing mutations. These results were validated for representative single amino acid substitutions expressed as individual, purified recombinant proteins. Conclusions: These data provide a useful resource for interpreting human mutations identified by future large scale clinical human genome sequencing. In addition, these findings provide new insight into structure-function relationships in PAI-1. Finally, these tools lay the groundwork for future studies aimed at developing novel SERPINs based on the PAI-1 scaffold with altered target protease specific potentially applicable to treatment for a number of disorders of hemostasis and thrombosis, as well as various other SERPIN disorders (eg alpha-1-antitrypsin and C1-inhibitor deficiency).