Microenvironment-dependent Models of the Proteolytic Network and Inhibition of Protease Activity

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Introduction: Proteases are central to many physiological functions, including protein turnover, immune-system response, and tissue remodeling. Unchecked proteolytic degradation can lead to disease through unintended degradation of cellular proteins. Proteases are tightly regulated and are considered as potential therapeutic targets for diseases ranging from cancer to osteoporosis; however, despite their promise as therapeutic targets, clinical trials have yet to bear fruit for some protease families, such as the cysteine cathepsins. In addition to regulation at the transcriptional level, protease-protease interactions regulate protease activity through proteolytic activation/degradation and these interactions define the collective proteolytic network. The existence of the proteolytic network can lead to non-intuitive responses to protease inhibitors, and we hypothesize these effects have contributed to the failure of cathepsin targeted therapeutics in the clinic. Cysteine cathepsins were originally thought of as lysosomal proteases, but have been found in the cytoplasm and acidified extracellular spaces in certain physiological/disease states, and as a result are found in multiple microenvironments. Favorability of interactions of the proteolytic network is affected by microenvironmental cues, such as pH; therefore, the direction and significance of connections within the proteolytic network, as well as, the expected system response to the addition of protease inhibitors, are dependent on where the protease is localized inside or outside the cell. Of particular interest to this work, is the proteolytic network defined by the cysteine cathepsin K, L, S, and V, which contains cathepsin "cannibalism" interactions, which lead to cathepsin degradation, that have been previously characterized by our group. The aim of the presented work is to develop computational models to predict the effect of the microenvironment on interactions of the cysteine cathepsin proteolytic network and to quantify the contribution of these interactions to the kinetics of substrate degradation. These models are aimed at better understanding the effects of perturbing the proteolytic network through the administration of protease inhibitors.

Materials and Methods: We have utilized a hybrid computational and experimental approach to elucidate the pH-dependence of cathepsin:cathepsin interactions and to quantify the contributions of these interactions to the dynamics of substrate proteolysis. We performed molecular modeling of cathepsins K, L, S, and V and their potential cross- and self-interactions to elucidate the effects of pH at the molecular-level. To investigate the effects of pH on the dynamics of cathepsin proteolytic network we have also developed systems-scale models of substrate proteolysis. *In vitro* experiments using recombinant enzymes were performed to elucidate the contribution of pH-dependent cathepsin:cathepsin interactions to cathepsin cannibalism, as well as, the effect of pH on substrate proteolysis in the context of the cathepsin proteolytic network.

Results and Discussion: Our molecular modeling indicates that specific cathepsin:cathepsin interactions form near predicted cleavage sites and that the favorability of the predicted complexes are highly dependent on pH. Experiments with recombinant enzymes show that cathepsin cannibalism is similarly dependent on pH, suggesting that the strong predicted cathepsin:cathepsin interactions lead to an increase in cannibalism. Our systems-scale models are able to predict the level of substrate proteolysis in presence of different combinations of the cysteine cathepsin, while simulations predict the effects of perturbing the cysteine cathepsin proteolytic network through the addition of cathepsin inhibitors.

Conclusions: The presented results suggest a potential role for cathepsin interactions as a microenvironment-dependent regulatory mechanism of cathepsin activity that should be considered in the design and administration of cathepsin targeted therapeutics. Additionally, the proposed hybrid framework lays the groundwork for further investigation of the role of microenvironmental pH in the regulation of the proteolytic network.