Cathepsin Cannibalism Reduces Collagen And Elastin Degradation In Matrix Remodeling

M. Ferrall[1] and M. Platt[1]
[1]Georgia Institute of Technology and Emory University, Atlanta, GA

Introduction: One of the classic "hallmarks of cancer" is the ability for cancerous cells to activate invasion and metastasis, sustain proliferative signaling, and evade growth suppressors. Matrix-degrading enzymes such as cysteine cathepsin proteases are involved with cross talk between cancer cells and neighboring stroma, which are recruited for invasion. Cysteine cathepsins include a family of 11 cathepsins, some of which are the most powerful human collagenases and elastases and are active in acidic environments. Of particular interest are cathepsins K, L, and S, which share 60% sequence homology and redundancy in target substrate proteins with different catalytic activities toward different ECM substrates, which have been investigated in isolation. However, this is not physiologically relevant as cells secrete many proteases simultaneously in the body. Previously, it has been shown that one species of cathepsin will preferentially degrade another while also in the presence of matrix proteins; which is termed cathepsin cannibalism. This work uses a computational model to probe the proteolytic network of cathepsin cannibalism between multiple species and multiple substrates. Such networks are difficult to study experimentally *in vitro*, so computational modeling is essential in shedding light on this mechanism.

Materials and Methods: Kinetic assays of fluorogenic substrates, which fluoresce when cleaved, with different combinations of recombinant cathepsins (cat) K, L, and S were preformed to determine the time series substrate degradation over 120 minutes. The model developed using a general mass action model fit the kinetic parameters of each cathepsins to the time series data of substrate degradation of different combinations of cathepsins.

Results and Discussion: We empirically obtained the parameters for the mass action rates through systematic analysis of fluorogenic gelatin/elastin degradation in the presence of multiple cathepsin species. The measured enzyme kinetics, in conjunction with local optimazation methods, were used to construct a mechanistic model of the possible interactions between cathepsins and substrates, as well as between cathepsin species. Cathepsin cannibalism allowed prediction of substrate degradation between pairs of catK, L, and S on gelatin and elastin. At an equal protease concentration, the preferential direction of cannibalism is independent of substrate; catS degrades catK and catL, while catK degrades catL. However, varying the concentration ratio between cathepsins regulates the cathepsin activity in the system, as shown in Fig. 1A, where catS reduces the activity of catK. Fig. 1B shows how the time to loss of catK activity (stars) is reduced by increasing the concentration of catS.

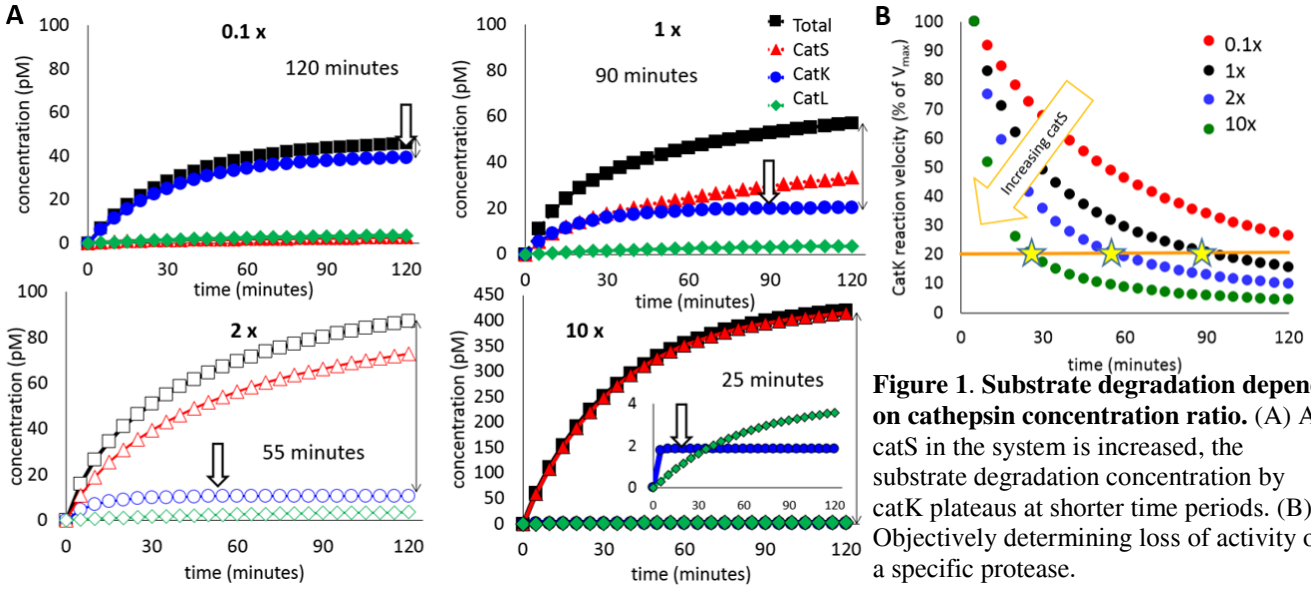

Figure 1. Substrate degradation depends on cathepsin concentration ratio. (A) As catS in the system is increased, the substrate degradation concentration by catK plateaus at shorter time periods. (B) Objectively determining loss of activity of a specific protease.

Conclusions: Here we have shown that multiple cathepsin species in a single system can, in fact, protect and preserve gelatin and elastin matrices due to cathepsin cannibalism, as observed between catK, L, and S. It is imperative to understand the consequences of having multiple proteases present develop clinically relevant interventions for cancer metastasis. To further validate these results, we are developing active-stie or cleavage-site mutant cathepsins that are resistant to cannibalism further confirm the preferential direction of cannibalism.

References: Hanahan D, *Cell*, 2011, 144, 646-674; Bromme D, *Biol Chem Hoppe Seyler*, 1995, 376, 379-84; Barry ZT, *J Biol Chem*, 2012, 287, 27723-30