Optimizing activation and purification for cathepsin proteases from engineered cell

Yuan Xu[1], William Shockey[1], Megan Ferrall-Fairbanks[1], and Manu Platt[1]
[1]Georgia Institute of Technology, Atlanta, GA

Introduction: Metastasis involves cellular invasion and migration, which is led by decline d amount of extracellular matrix (ECM). Malignant tumors express excess proteolytic proteins, which serve the purpose of digesting ECM. Cysteine cathepsin proteases contribute to the degradation of intracellular and extracellular proteins, which contributes to remodeling ECM and inducing tumor growth and metastasis. Cathepsin cannibalism is an interaction that occurs when active cathepsins concentrations decline and reduce substrate degradation in the biological system. In order to research the proteolytic network of cysteine cathepsins and extracellular proteins, our lab has previously designed mutant cathepsins that will shift the proteolytic network, but they need to be tested for their kinetic properties. To do so, these enzymes must be expressed, purified, collected and activated for in vitro studies. Mostly bacterial and yeast systems were used for recombinant expression, but we seek to do this in eukaryotic cells to ensure post-translational modifications that would be found in human cells during human disease progression occur. Cathepsins are translated in their immature form, pro-cathepsin, which contains pro-peptide at the active site. Cathepsin activation requires cleavage of pro-peptide, which exposes the active site for cathepsins to become mature. The objective of the experiment is to determine the optimal activation method for pro-cathepsins to mature cathepsins after purification from cathepsin-secreting-engineered cell lines.

Materials and Methods: Cathepsin K, L, S were extracted from HEK 293T and RAW 264.7 murine macrophages and activated using an activation method from previous publication, dextran sulfate activation. Zymography and western blot were used to determine the active amount and expression of active cathepsin. Zymography separates proteases through gelatin embedded SDS-PAGE gel, then proteases recover activity through incubation. Active proteases digest embedded gelatin at position, which provides zymography signals.

Results: Cathepsins are purified and concentrated from cell media for activation and protein assay (Figure 1A). Purification process successfully extracted cathepsin from cell media (Figure 1B) using nickle chelating resin column (Geno). However, cathepsins extracted were shown not active by zymography (data not shown). Therefore, different cathepsin activation methods were tested. Among methods tested, dextran sulfate activation was able to provide strong signals. Dextran sulfate is an artificial glycosaminoglycan, which is a molecule found in the ECM known to induce cathepsin activity (Dejan Caglic. 2007). Zymography shows strong signal after activation from cathepsins (Figure 1C). Therefore, dextran sulfate activation method can be used on transfected HEK 293T cells for recombinant cathepsins.

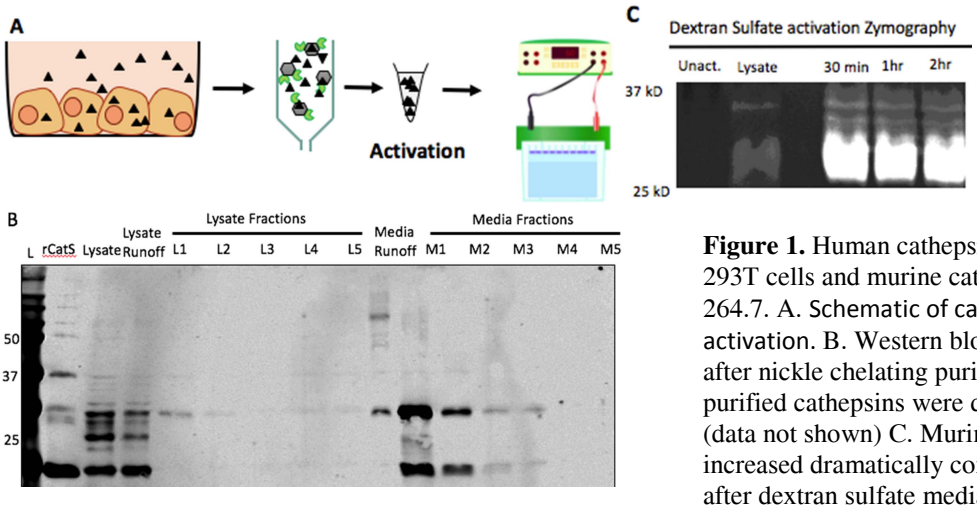

Figure 1. Human cathepsins extracted from HEK 293T cells and murine cathepsins activated from 264.7. A. Schematic of cathepsin purification and activation. B. Western blot shows cathepsin present after nickle chelating purification. However, no active purified cathepsins were detected on zymography (data not shown) C. Murine cathepsins activity signals increased dramatically comparing to positive controls after dextran sulfate mediated activation.

Conclusions: Results from zymography suggest that dextran sulfate mediated activation successfully activates cathepsins from RAW264.7 murine macrophage cell line. For future experiments, dextran sulfate activation will be used for activating wild type cathepsins from transfected HEK 293T cells for kinetics studies.