UC San Diego
MOORES CANCER CENTER
An NCI-designated Comprehensive Cancer Center

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"Global Profiling of Extracellular Protease Activity from Lung Cancer Cells"

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One of the hallmarks of aggressive cancer is its ability to spread to new tissues, a process that is mediated in part by the activity of extracellular proteases. In healthy cells, protease activity is tightly regulated by subcellular localization, the presence of endogenous protease inhibitors, and requisite conversion from inactive precursor forms. However, dysregulated protease activity is evident in many cancers, including lung cancer. We have developed a novel mass spectrometry-based screening strategy that can identify the global substrate specificity and kinetic efficiency of proteases alone and in complex biological mixtures by employing a small, diverse library of rationally designed peptide substrates. This assay, referred to as Multiplex Substrate Profiling by Mass Spectrometry (MSP-MS), marks a significant advance in protease profiling by allowing for the unbiased and simultaneous detection of all protease activities in a given sample. We have successfully applied the MSP-MS assay to measure total protease activity in the secretions of breast cancer cell lines and detected a wide range of amino peptidases, endo-proteases and carboxypeptidases. Comparative analysis of conditioned media from different breast cancer cell lines revealed distinct differences in proteolysis between invasive and non-invasive cells.

Specific Aims

Specific Aim 1. Identification of Proteolytic Enzymes in Conditioned Media from Cell Culture Models of Lung Cancer

Specific Aim 2. Profiling Global Extracellular Protease Activity in Lung Cancer Cell Lines

The goal of Specific Aim 1 is to identify all proteases in the secretome of lung cancer cell lines. To achieve this we will generate conditioned media from twelve lung cancer cells lines representing small cell carcinoma, adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Proteins will be identified using an Orbitrap Fusion Tribrid Mass Spectrometry, the most advanced instrument at UCSD. For Specific Aim 2, we will uncover the global protease substrate specific profile of the conditioned media from each of the lung cancer cell lines. Incorporation of class-specific protease inhibitors into the multiplex assay, will be used to determine the contribution of individual enzymes to the overall substrate specificity profile. For example, if a cysteine protease is identified in media, assays will be performed and in the presence and absence of a cysteine protease inhibitor (e.g. E-64).

In collaboration with the Craik lab at UCSF, we have used the MSP-MS method to assay cyst fluid to differentiate benign lesions from aggressive pancreatic cancer. Therefore, once we have established which proteases are secreted by lung cancer cells, a downstream goal would be to assay lung pleural fluid or conditioned media from primary lung and metastatic tumor cultures. In the longer-term, we

predict that uncovering the global profiles of extracellular protease activity in lung cancer may identify therapeutic targets or facilitate the development of protease-activatable imaging agents or protease-activatable pro-drugs.