Profiling protease activity in acute lymphoblastic leukemia with chemical probes

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Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, originating in the bone marrow the primary site of blood cell production and maturation. In ALL, malignant cells replace healthy hematopoietic precursors, impairing marrow function and disrupting hematopoiesis. Diagnosis relies on bone marrow aspiration supported by immunological and cytogenetic profiling. Despite cure rates exceeding 90%, about 10% of pediatric patients remain resistant to standard therapies. In this project, we investigate mechanisms of treatment resistance by analyzing proteolytic enzymes implicated in leukemia progression, including MALT1, cathepsins S, B, L, legumain, ADAM10, and the 20S proteasome. Conventional fluorescent activity probes assess only single enzymes and lack the capacity for multiplexed single-cell analysis. To overcome this limitation, we develop lanthanide-labeled probes based on selective peptide inhibitors of individual proteases. These peptide sequences form the basis of a theranostic platform: they can function either as diagnostic probes tagged with stable metal isotopes for enzyme activity profiling, or be converted into prodrugs specifically activated by the same proteases. Mass cytometry is an advanced single-cell technique widely used in immunology and oncology to analyze over 50 biomarkers simultaneously via distinct lanthanide isotopes, enabling detailed characterization of cellular subsets, functional states, and protein expression. However, conventional approaches are limited to measuring protein levels and cannot directly report enzyme activity. Currently, these probes are being tested in leukapheresis samples for optimization, with the aim of future application in bone marrow. By integrating lanthanide-tagged probes with mass cytometry, we enable multiplexed monitoring of proteolytic enzyme activities in individual leukemia cells.