Application of metal-tagged TOF probes for proteases in breast cancer using imaging mass cytometry

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Proteases are known to be among the key players in modulating the tumor microenvironment. Therefore, to gain a better insight into such a complex cellular landscape, we decided to use mass cytometry, which uses antibodies conjugated with stable metal isotopes. The excellence of this method lies in the fact that each isotope has its peak on the mass spectrum, eliminating signal overlap and consequently enabling the monitoring of more than 50 parameters with a single cell resolution. Mass cytometry enables the analysis of cells in suspension and as a tissue imaging system - Imaging Mass Cytometry (IMC), providing comprehensive information about tissue architecture and its microenvironment. Since antibodies allow detection of the total amount of enzymes, we postulate that this approach requires a more sophisticated attitude, like using activity-based probes (ABPs). The undoubted advantage of using ABPs is the detection of only active enzymes. In our laboratory, we are adapting ABPs for mass cytometry purposes. The first demonstration of the technology was successfully conducted, using PBMC from a healthy donor [1]. We are now trying to extend the technology to use the same probes in IMC. In this study, we use metal-labeled antibodies for cancer architecture along with protease antibodies, enzymes inhibitors and corresponding ABPs to stain breast cancer tissues. This approach allows us to detect the localization and activity pattern of selected cancer-associated proteases. Our approach might bring many benefits to the field of personalized cancer diagnosis and treatment, as proteases contribute to anticancer chemo- and immunotherapy resistance. Moreover, our approach might be directly used for the development of highly selective antibody-drug conjugates that are activated by proteases displaying elevated activity.

[1] Poreba, Marcin et al. “Multiplexed Probing of Proteolytic Enzymes Using Mass Cytometry-Compatible Activity-Based Probes.” *Journal of the American Chemical Society* vol. 142,39 (2020)

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