High selectivity single-cell protein assays enabled by microfluidic design

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Dysregulation of signaling pathways is a hallmark of diseases including cancer. Cell-to-cell variation in this dysregulation is a critical aspect of disease development and progression. While single-cell resolution genomic and transcriptomic tools are advancing, these nucleic acid measurements do not directly measure protein-mediated signaling including dynamic post-translational protein modifications, protein localization, and protein-protein interactions. A majority of cancer therapies target proteins. Further, tumoral heterogeneity drives both drug response and resistance. Single-cell resolution protein tools can provide new insights.

Powerful single-cell protein analysis tools do exist, yet all contemporary approaches to detect unmodified endogenous proteins are immunoassays (e.g., IHC/ICC, flow, and mass cytometry, and various ELISAs including microfluidic tools). Yet, the analytical specificity of immunoassays is dictated by immunoreagents (e.g., antibodies) which are notorious for sub-optimal performance. Limited availability of some immunoreagents severely curtails multiplexing capacity (e.g., off-target background signal) and the range of protein targets (especially isoforms). To mitigate these shortcomings of immunoassays, researchers prepend an electrophoretic protein separation to the immunoassay, thereby loosening constraints on immunoreagent performance and enhancing assay specificity. The tandem protein separation and immunoassay is known as a western blot. Unfortunately, the conventional western blot requires pooling of thousands of cells for each measurement, thus obscuring important cell-to-cell variation in protein expression and state.

We recently developed a microfluidic approach to achieve single-cell resolution western blotting, thus enabling direct measurement of proteins in single cells when high selectivity is required or when starting cell populations are exceedingly small. This presentation will discuss both fundamental and applied aspects of the targeted proteomics tools. In fundamentals, we will detail how a combination of separations science, microfluidics, and polymer formulation contributions offers a single-cell western blot with high throughput (>6,000 concurrent single-cell protein assays). In applied aspects, the presentation will highlight biological studies made possible by high selectivity protein measurements, including as related to drug resistance development in breast cancer, circulating tumor cells as indicators of cancer disease state, and as a tool to monitor sub-cellular localization of proteins as is relevant to IRES trans-acting factors. Looking forward, we see single-cell resolution targeted proteomics as an important complement to nucleic acid measurements and discovery proteomics.