Measuring Kinase Activities in Single Cells

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***Short Abstract* — Understanding the relationship between the internal signaling state of the cell in response to environmental cues and the resulting cellular phenotype is key for understanding how cells function, both normally and in disease states. We present a microfluidic device that allows detection of multiple kinase activities from individual adherent cells grown in standard tissue culture. This assay method will provide access to a clearer picture of cell signaling state and help to clarify the mapping between signals and cellular outcome.**

In order to understand the normal functioning of cells as well as disease states, it is crucial to understand the relationship between the internal signaling state of the cell (for example, the activities of kinases) in response to environmental cues and the resulting phenotype. Measurements made on many cells in bulk, as is done with many traditional assays (such as Western blotting), obscure differences that may exist between individual cells within a population, potentially blurring the picture of cellular signaling state.

A previously designed microfluidic concentrator device allowed detection of kinase activity from amounts of cell lysate equivalent to that of a single cell [1], using peptide probes exhibiting chelation-enhanced fluorescence [2]. We have developed a PDMS micropipet that allows lysis of single cells from adherent culture and collection of the single-cell lysate for assay with these kinase probes. The direct measurement of activity is a significant advantage over using levels of phosphorylated kinase as a proxy, as it has been shown that phosphostate is not always representative of activity [3]. The assay also affords the advantage of not requiring genetic manipulation that could affect the system under study.

We demonstrate sufficient sensitivity to measure MAP kinase-activated protein kinase 2 (MK2) activity from a single hepatocellular carcinoma (HepG2) cell stimulated with hyperosmotic shock [4]. We additionally demonstrate the ability to simultaneously measure the activities of multiple kinases (MK2, Akt, and PKA) along with a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) loading control from an individual cell. Using this device, we aim to allow assessment of heterogeneity in a cell population and access to a clearer picture of cell signaling state. This assay, with its compatibility with standard adherent cell culture platforms, will allow near-concurrent phenotypic and signaling measurements to be made on the same individual cell, providing a clearer connection between signals and cell outcome.

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