A rapid screening method for monitoring signaling changes in the monocyte cell line U937

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Abstract

Here we describe a precise screening assay that quantifies changes in phosphorylation of proteins in samples from as few as 100 cells, which is simple, rapid and relatively low in cost. A nano-immunoassay system (Cell Biosciences) was used to measure changes in expression and activation of relevant signaling proteins, including MEK, ERK and STATs in U937 monocyte cells before and after cytokine treatment. A single pan-specific antibody was used to distinguish between the phosphorylated and non-phosphorylated protein isoforms, as the nano-immunoassay (NIA) method separates different phosphorylated forms of a protein based on their isoelectric point. In parallel, phospho-protein FACS analysis, which is the current state of the art for measuring multiple signaling pathways, was performed to compare changes in expression and phosphorylation of the signaling proteins. Phospho-protein FACS analysis is expensive and requires considerable technical expertise, which limits its application for large numbers of samples. This novel nano-immunoassay screening method is currently being employed at the Stanford Human Immune Monitoring Core (HIMC) and is being used for high-throughput screening of compounds that influence monocyte activation, monocyte/macrophage differentiation and analysis of various disease states in small primary tissue samples. Practical examples will be given.

Introduction

Rapid screening assays for compounds affecting immune function are limited to proliferation assays and phospho-flow cytometry.

These techniques are expensive and require a high degree of technical ability to run and interpret results after an assay has been optimized.

The alternative assay is western blot which are labor intensive, semi-quantitative and relatively low throughput.

We describe the use of a non-immunoassay that can be used on lysates of U937 cells to measure charge variation in signaling proteins that correlates well with phospho-flow and western blot techniques.

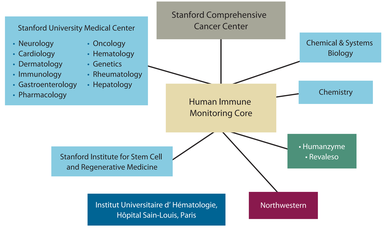
This NIA can be scaled, is extremely economical to run and can also be run on clinical samples.

Experimental Goals

Validate that NIA can measure changes in activation of key proteins such as p-ERK and p-MEK in U937 monocyte cell line after stimulation with various cytokines.

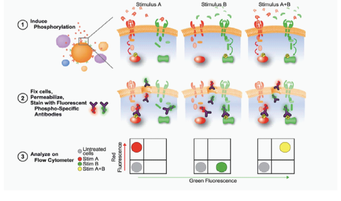
Validate that measurements correlate well with phospho-flow analysis assays.

HIMC Philosophy

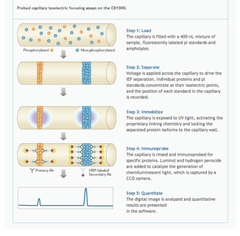


Technologies Utilized

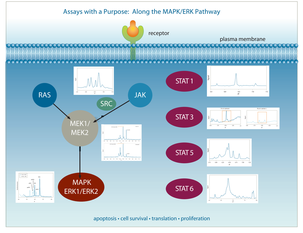
General phospho-protein staining technique for flow cytometry

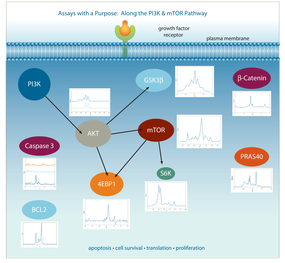


NIA Protocol



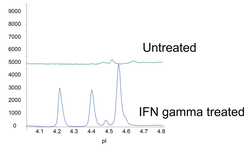
Signaling Pathway Targets

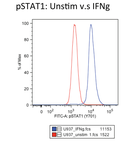




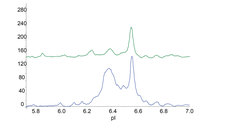
STAT Proteins

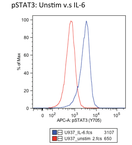
Increase of pStat1 in U937 cells by IFN gamma



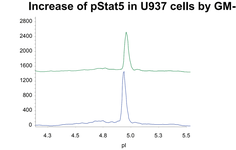


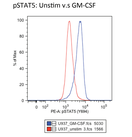
Increase of pStat3 in U937 cells by IL-6



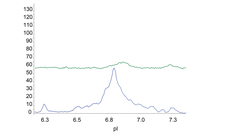


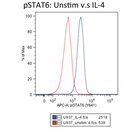
Increase of pStat5 in U937 cells by GM-CSF



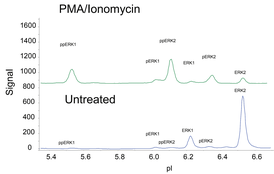


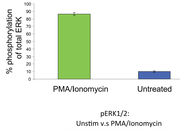
Increase of pStat6 in U937 cells by IL-4

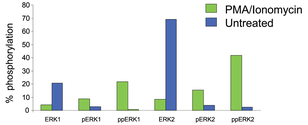


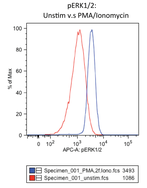


MAPK Pathway - ERK

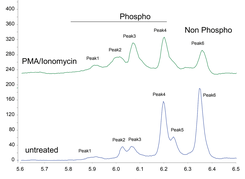


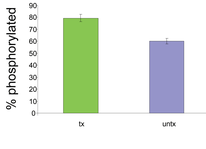






MAPK Pathway - MEK





Conclusions

Established U937 monocyte cell line as a screening model for cell signaling changes.

This model can be used for screening of novel reagents to measure new signaling pathways.

Previous findings of ERK signaling by phospho-flow were confirmed and are being extended to other MAPK signaling nodes; namely MEK signaling effects reported here.

This method allows rapid evaluation of large numbers of patient samples for differences in mitogenic inhibitory molecules.