MICROFLUIDIC IMAGE CYTOMETRY (MIC) TECHNOLOGY FOR *IN VITRO* MOLECULAR DIAGNOSTICS

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ABSTRACT

Microfluidic image cytometry (MIC) technology has been developed and utilized to perform quantitative/multiparameter immunocytochemistry (ICC), enabling parallel detection of multiple proteins in individual cells. A number of glioblastoma cell lines, as well as genetically modified and primary glioblastoma cells were utilized to validate the MIC technology. The dynamic changes of the proteins associated with the PI3K signaling pathway upon the exposure to exogenous stimuli (e.g., growth factors and kinase inhibitors) can be kinetically monitored, providing a potent tool to better understand how these glioblastoma cells respond to a combination of drug treatments.

KEYWORDS: Immunocytochemistry, Signaling Profile, Image Cytometry, Cancer Diagnostics

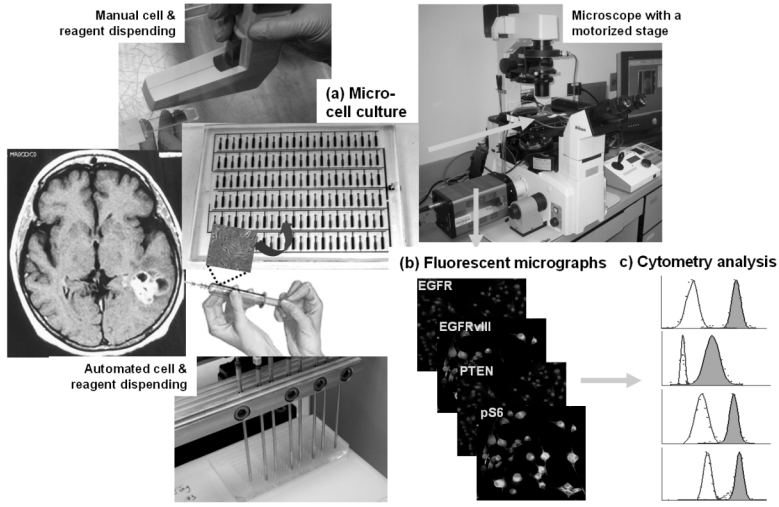

Figure 1. The basis of the MIC technology is the integration of (a) long-term microcell culture of cancer cells, (b) simultaneous fluorescent imaging of multiple probes at a single cell level, and (c) reproducible cytometry analysis.

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INTRODUCTION

Alterations in intracellular signaling networks promote the proliferation and invasion of cancer cells. A systems-oriented approach to cancer aims to define multiple signaling proteins in the networks that drive the malignant transformation of cancers, thus necessitating a technology platform capable of detecting multiple proteins in tumor samples. Since tumor samples are composed of mixtures of cancer, stromal, vasculature and immune cells, such a technology platform also requires single-cell precision in order to capture the nature of cellular heterogeneity. As a first step towards creating a technology for the profiling of cancer signaling networks, our research group has developed MIC technology (Figure 1), based on a microfluidic cell array in conjunction with an automated (or a semi-automated) pipette (for cell loading, fixation, permeabilization and ICC) and a fluorescence microscope (for imaging acquisition and cytometry analysis).

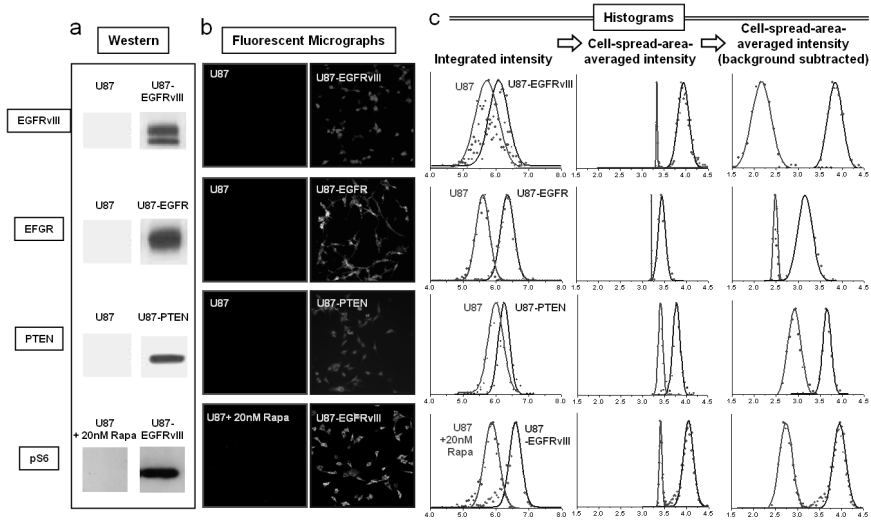

Figure 2. Dynamic ranges of the MIC technology for single-cell profiling of EGFRvIII, EGFR, PTEN and pS6. a) Western data for detecting the four signaling proteins. b) Fluorescent micrographs of the ICC-treated cells. c) The dynamic ranges for quantification of the four signaling proteins in individual cells.

RESULTS AND DISCUSSION

A number of glioblastoma brain cancer cell lines (i.e., U87 and LN229 cell lines), as well as genetically modified and primary glioblastoma cells were utilized to validate the MIC technology. The expression/phosphorylation levels of the four signaling proteins associated with the PI3K-AKT-mTOR pathway, including receptor tyrosine kinases (EGFR and EGFRvIII), phosphotase (PTEN) and phosphorylated protein (pS6) can be quantified in parallel with single-cell precision (Figure 2). MIC technology is capable of parallel detection of several signaling molecules in individual cells, thus a collective molecular signature of individual cells can be utilized to capture the nature of cellular heterogeneity in tissue samples. Artificial cell mix-

tures and surgically removed tumor tissues were utilized to demonstrate how MIC-based multiparameter analyses can be utilized to distinguish different cell types. Remarkably, the dynamic changes of those proteins upon the exposure to exogenous stimuli (e.g., growth factors and kinase inhibitors) can be kinetically monitored, providing a potent tool to better understand how these glioblastoma cells respond to a combination of drug treatments. Rapamycin is a potent inhibitor targeting mTOR – a critical signaling molecule involved in the PI3K signaling pathway. The effect of rapamycin inhibition on mTOR can be quantified by monitoring the activation/phosphorylation of the downstream signaling molecule pS6. MIC technology was used to quantify rapamycin-induced down-regulation of pS6 levels in individual cells and IC50 was found at 2.69 nM (Figure 3).

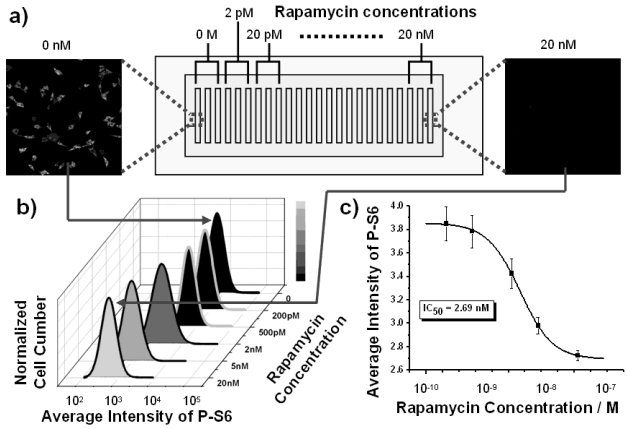

Figure 3. Quantification of pS6 expression levels in individual cells in the presence of different concentrations of rapamycin. a) U87 cells were cultured in a microfluidic cell array chip with different concentrations of rapamycin. b) The rapamycin-induced down-regulation of pS6 levels were quantified by the MIC technology. c) A dose-dependent curve was obtained.

CONCLUSIONS

We envision that the MIC technology will be able to achieve: i) dramatic improvement in speed, accuracy and fidelity of cancer diagnosis, ii) reduced cancer sample consumption, iii) provision of signature identification to guide the implementation of targeted therapies, iv) a perfect conjunction with the needle biopsy operation, v) greater cost efficiency compared to traditional methods and other state-of-the-art technologies, and vi) broad application to cancers in other organ systems.

ACKNOWLEDGEMENTS

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