Simultaneous quantitation of multiple signaling molecules in individual Circulating Tumor Cells (CTCs) by multi-color laser scanning cytometry

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ABSTRACT (#4155)

Introduction: Recent advances in circulating tumor cell (CTC) technologies provide a new alternative approach to examine proteomic and molecular characteristics of tumor cells from cancer patients. The current trend in anti-cancer drug development focuses on new compounds that modulate multiple signaling pathways. CTC detection is being increasingly used as a liquid biopsy, complimentary to tumor biopsies, and the simultaneous examination of signaling pathways in CTCs at the single cell level can provide insight into drug activity in tumor cells. We used proprietary laser scanner cytometry (LSC) technology to developed a multi-color immunofluorescence (IF) procedure to detect multiple biomarkers (level of expression and localization) within the same CTC. We validated this new methodology using (1) cell lines treated with or without a DNA-damaging agent or receptor ligand stimulation, and (2) a simultaneous 6-color detection of maker expression. **Purpose:** To develop a method that combines multiple markers IF staining with LSC analysis to characterize CTCs. **Procedures:** A target biomarker staining panel was combined with standard CTC staining (DAPI, CK and CD45) to create a 6-color IF staining panel to examine marker expression in tumor cell lines by LSC. **Results:** Using a simultaneous co-staining procedure and LSC-based analysis, we demonstrated that treatment of A549 cells with bleomycin induced translocation of p53, treatment of LNCaP cells with mibolerone induced AR translocation, and treatment of MCF-7 cells with IGF-1 induced IGF-1R phosphorylation, illustrating that different types of functional markers can be examined by LSC. **Summary** A multi-color, LSC-based methodology was developed to quantify expression of up to at least 3 biomarkers in CTCs at the single cell level. Application of this technology in clinical studies to examine CTCs from patients could provide important information on pathway-specific drug activity and guide dosing selection.

MATERIALS AND METHOD

Cell lines

Lung cancer cell line A549, breast cancer cell lines MCF7 and SKBr3, and prostate cancer cell line LNCaP were used for drug treatment and protein expression analysis.

Drug treatment

- A549 cells were treated with Bleomycin to induce p53 translocation
- LNCaP cells were treated with Mibolerone to induce AR translocation
- MCF7 cells were treated with IGF1 to induce IGF-1R phosphorylation

LSC analysis of protein expression in CTCs

Cells were stained with combinations of biomarker-specific antibodies and subjected to immunofluorescent analysis of protein expression using multicolor LSC. Immunofluorescent staining was complemented by antibodies against cytokeratin and CD45 to insure unambiguous identification of $CK^+/CD45^-/DAPI^+$ CTCs enriched from cancer patient blood.

RESULTS

Upregulation and nuclear translocation of p53 induced by treatment of A549 lung cancer cells with bleomycin

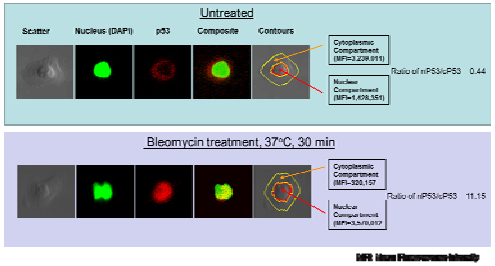

Figure 1. The wild-type p53 lung cancer cell line A549 was treated with bleomycin at 50 mU/ml or PBS for 15 minutes, cytospun onto glass slides, fixed, permeabilized, stained with anti-p53 antibody and analyzed by LSC.

IGF-1 treatment upregulates IGF-1R receptor phosphorylation in MCF-7 breast cancer cells

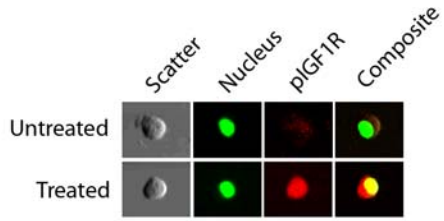

Figure 2. Pre-treatment of MCF7 cells with IGF-1 for 15 min led to an increase in anti-phospho-IGF-1R staining, indicating that the selected antibody can detect ligand-induced changes in phospho-IGF-1R levels within cancer cells.

Upregulation and nuclear translocation of AR induced upon LNCaP prostate cancer cell treatment with mibolerone

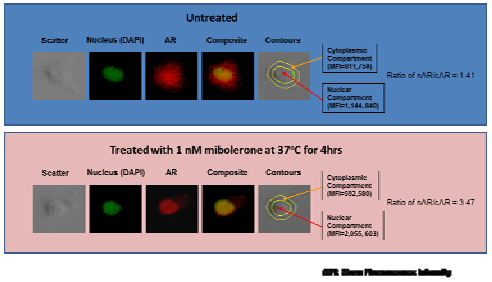

Figure 3. LNCaP cells were treated with 1 nM mibolerone, an androgen agonist, for 15 minutes. Cells were fixed, permeabilized, and stained with anti-AR antibody.

Simultaneous quantitation of multiple signaling molecules in SKBr3 breast cancer cells

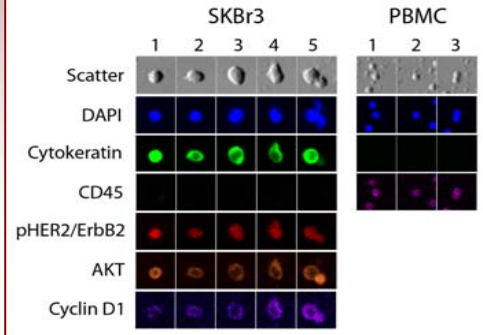

Figure 4. SKBr3 cells were cytospun onto glass slides, fixed, permeabilized, and stained simultaneously with antibodies for CK, CD45, pHER2/ErbB2, AKT, and cyclin D1. DAPI was applied to the cells for nuclear staining prior to LSC analysis. PBMCs isolated from normal donor blood were stained with cytokeratin and CD45 antibodies and DAPI to demonstrate negative CK and positive CD45 staining.

CONCLUSIONS

- We used laser scanning cytometry to detect drug-induced protein translocation or ligand-induced protein receptor phosphorylation in various cancer cell lines.
- We developed an IF staining procedure to simultaneously examine up to 3 protein markers within CTCs identified by cytokeratin, CD45 and DAPI staining.
- Application of integrated CTC isolation and LSC analysis technologies in clinical studies could provide important information on pathway-specific drug activity as well as guide dosing selection.