Proprietary High Content Imaging and Characterization of Circulating Tumor Cells from Cancer Patients

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Background

Recent advances in circulating tumor cell (CTC) technologies provide a new approach on proteomic and molecular characterization of tumor cells from cancer patients. The absolute CTC count by the current FDA cleared CellSearch system, in most cases, remains low, which makes further CTC analysis challenging. Here we demonstrate an improved, highly sensitive approach for CTC enumeration and protein expression based on the CellSearch CTC Profile Kit and high-content, laser scanning cytometry (LSC) technology. We developed a multi-color immunofluorescence procedure to detect protein expression and localization of multiple biomarkers in individual CTCs. We further examined genetic alternations by fluorescence in situ hybridization (FISH) using a relocating image system.

Results

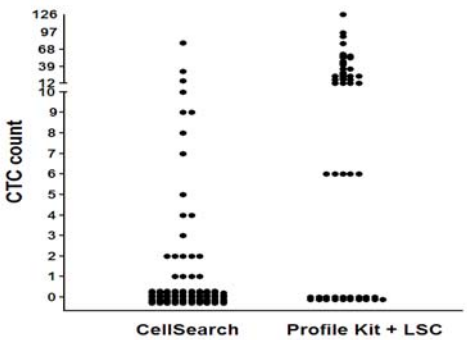
Side-by-Side Comparison between CTC recovery by Profile Kit/LSC Method and Standard CTC Enumeration Kit

CTC recovery from blood of the same cancer patients was performed using both CellSearch Profile Kit/LSC and CellSearch CTC Kit. Profile Kit/LSC method recovered more CTCs as compared with CellSearch CTC kit.

Patient ID	Primary Diagnosis	Count Per 7.5mL Blood	
		CellSearch® Profile Kit + LSC	CellSearch® CTC Kit
1	HCC	25	0
2	HCC	97	0
3	HCC	27	0
4	Prostate cancer	88	52
5	Prostate cancer	178	48
6	Prostate cancer	47	0
7	Prostate cancer	31	0
8	Prostate cancer	79	1
9	Prostate cancer	0	4
10	Prostate cancer	43	0
11	Prostate cancer	127	1
12	Prostate cancer	75	1

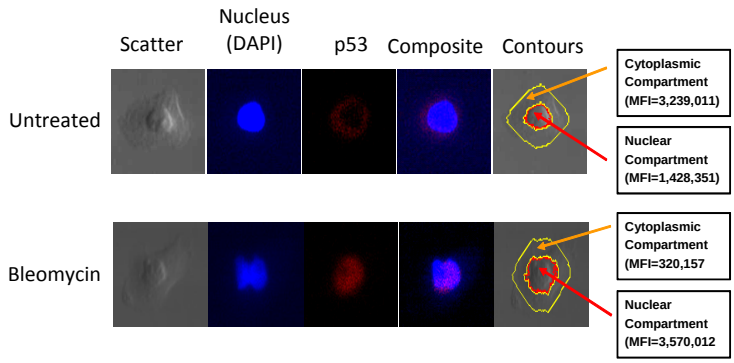
Overall Comparison between CTC Recovery by Profile Kit/LSC Method and Standard CTC Enumeration Kit

CTC recovery in 90 cancer patients by CellSearch CTC Kit was compared with CTC recovery in 52 cancer patients by Profile Kit/LSC method.



Profile Kit/LSC method recovered a significantly larger number of CTCs compared with CellSearch CTC Kit ($p<0.01$). 69 out of 90 (77%) were CTC-negative with standard CTC kit, while only 37% of patients were CTC-negative by Profile Kit/LSC method.

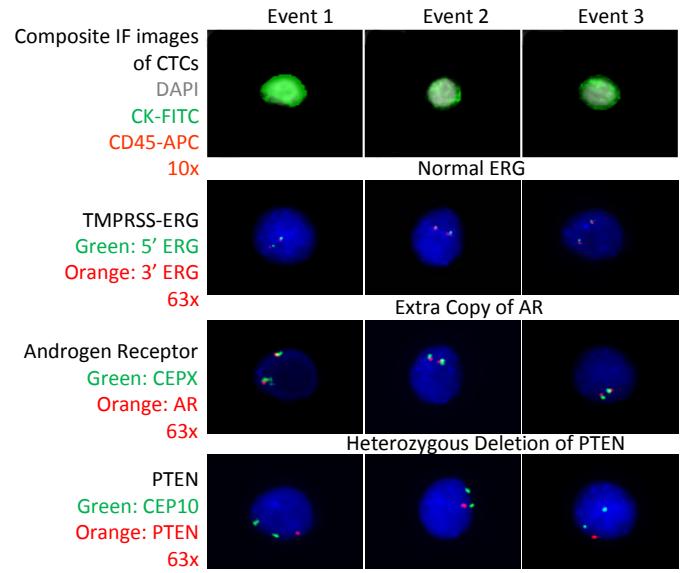
Nuclear Translocation of p53 upon Exposure of A549 cells to Bleomycin



A549, a lung cancer cell line, was treated with DNA-damaging agent, bleomycin, at 37°C for 30 min (lower panel), or left untreated (upper panel). Cells were fixed, permeabilized and stained with anti-p53 antibody. Translocation of p53 into nucleus upon cells' exposure to bleomycin was detected by a laser scanning cytometry (Compucyte).

(MFI, mean fluorescence intensity).

FISH Detection of TMPRSS2-ERG, AR and PTEN in Prostate Cancer CTCs Demonstrates AR Gain and Heterozygous Deletion of PTEN in the Same CTCs



Conclusions

- A proprietary process platform integrating the CellSearch Profile kit (to increase CTC recovery) and LSC (to collect high-content marker information) provides a powerful tool to characterize CTCs collected from cancer patients.

- The incorporation of these technologies into clinical studies may help to identify molecular mechanisms of action and facilitate patient stratification for more effective therapies.