Abstract 750

Detection of mutations in single circulating tumor cells using MALDI-TOF mass spectrometry

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Background

As cancer therapeutics are increasingly selected based upon molecular genetic information, having reliable, accurate, rapid and inexpensive methods for mutational analysis are extremely desirable. Circulating tumor cells (CTCs) allow non-invasive "liquid biopsy" access to intact cells for molecular analysis. Here we demonstrate the successful detection of mutations in model CTCs individually isolated from blood (AccuCyte® - CyteFinder® system, RareCyte) using MALDI-TOF Mass Spectrometry MassARRAY® technology, Agena Bioscience).

Study Design

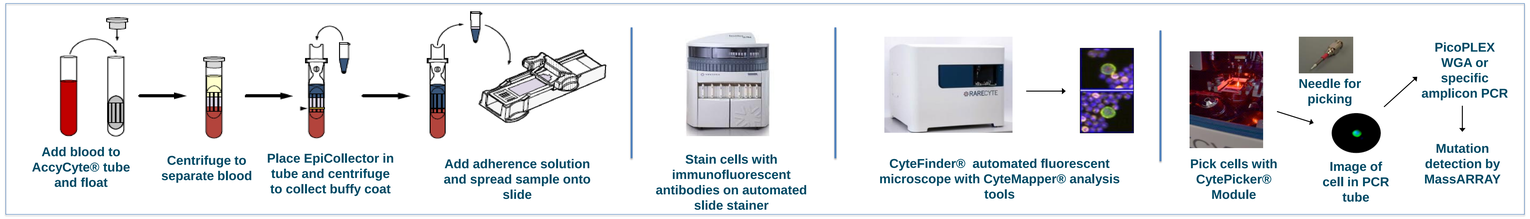
Breast (MDA-MD-231) and lung (NCI-H1975) cancer cells with a set of known mutations were spiked into blood and processed by AccuCyte onto microscope slides and stained on an automated immunostainer. Slides were imaged using the CyteFinder digital fluorescence scanning microscope and mCTCs were identified by positive nuclear, EpCAM, and cytokeratin staining, and negative CD45 staining. mCTCs and white blood cell (WBC) negative controls were picked from the slides and put into PCR tubes using the CytePicker® module. DNA from individual or small pools of cells (3-5) was amplified using the PicoPLEX® (Rubicon) whole genome amplification (WGA) kit; alternatively cells were lysed and directly entered into the ensuing iPLEX® Pro workflow. Specific regions surrounding 5 different mutations in each of the mCTC lines were amplified from the WGA product or the lysed cells and the products were detected and scored for the mutations using a single PCR reaction iPLEX® Pro panel that includes a combination of 10 common lung and breast cancer mutations using the MassARRAY® platform.

Results

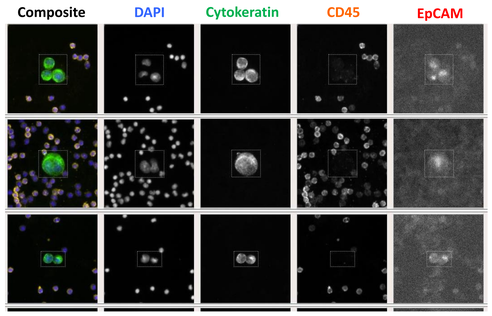
Five point mutations in four different genes (*CDKN2A, EGFR, PIK3CA*, and *TP53*) were measured in the NCI-H1975 lines and four point mutations in four genes (*BRAF, KRAS, NF2*, and *TP53*) and a deletion in one gene (*CDKN2A*) were measured in the MDA-MB-231 cells by iPLEX® Pro chemistry on the MassARRAY® System. All mutations were accurately detected in the WGA single and pooled cell samples and most were also detected in cells that did not undergo WGA before PCR with the iPLEX® Pro panel.

Table of mutations targeted for SNP genotyping

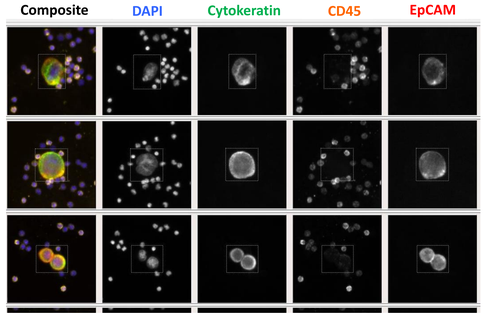
Cell Line	Tumor	Gene	Mutation	Zygosity
NCI-H1975	NSCLC	CDKN2A	c.205G>T	homozygous
		EGFR	c.2369C>T	heterozygous
		EGFR	c.2573T>G	heterozygous
		PIK3CA	c.353G>A	heterozygous
		TP53	c.818G>A	homozygous
MDA-MB-231	Breast	BRAF	c.1391G>T	heterozygous
		CDKN2A	c.1_471del471	homozygous
		KRAS	c.38G>A	heterozygous
		NF2	c.691G>T	homozygous
		TP53	c.839G>A	homozygous



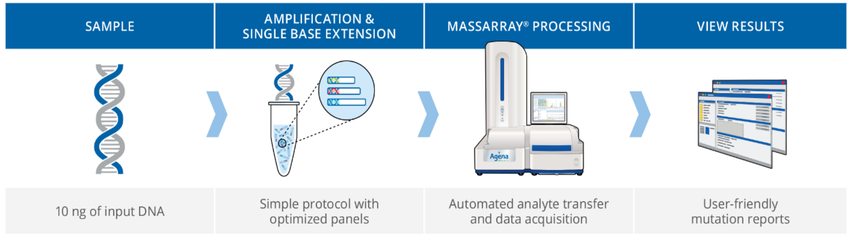
MDA-MB-231 cells identified from spike-in



NCI-H1975 cells identified from spike-in



WORKFLOW - Somatic mutation detection



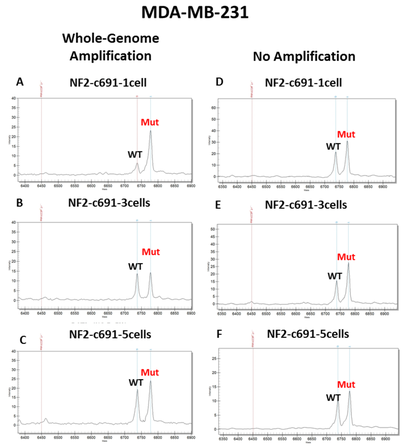
DNA to data in ~8 hours with less than 30 minutes of manual processing time.

Schemtic workflow for somatic mutation detection using iPLEX® chemistry and the MassARRAY® System

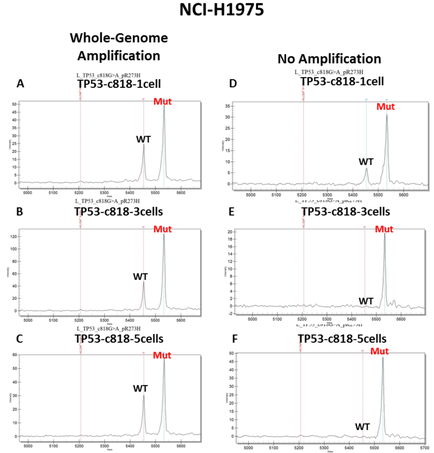
SNP Genotyping

Genotyping of SNPs was performed using IPLEX® Pro chemistry on the MassARRAY® System (Agena Bioscience., San Diego, CA, USA), that employs matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as the amplicon detection system (MALDI-TOF-MS; SpectroACQUIRE, Agena Bioscience). Primers designed for PCR (polymerase chain reaction) amplification of specific mutations (see table): Taq DNA polymerase, genomic DNA (10-15ng), PCR primers and dNTP. Following PCR (45 cycles), the remaining dNTPs were removed by the addition of alkaline phosphatase, after which the plates were incubated at 37°C for 40 min. Extension reactions were prepared using the MassARRAY® Assay Design Version 3.1 (Agena Bioscience., San Diego, CA, USA). Single based extensions of the terminator signal in a DNA specimen can be reliably detected by the MassARRAY® System. Finally, the samples were desalted by resin treatment for 15 min, spotted into SpectroCHIP® Arrays (Agena Bioscience., San Diego, CA), analyzed by mass spectrometer and ultimately interpreted on TYPER 4.0 software (Agena Bioscience., San Diego, CA).

Examples of mutations found in single circulating tumor cells



Comparison of Mutation NF2-c691 from MDA-MB-231 cells from single cell (1cell) extraction or pooling of cells (3 cell or 5 cells). Spectra were obtained using iPLEX® Pro chemistry on the MassARRAY® System. Location of wild type (**WT**) and mutant allele (Mut). **A-C** Spectra of 1, 3 and 5 cell pools were from samples that underwent both whole genome amplification and the iPLEX® Pro assay. **D-F** Spectra of 1, 3 and 5 cell pools that did not go through whole genome amplification but were only processed using standard iPLEX® Pro PCR amplification.



Comparison of Mutation TP53-c818 from NCI-H1975 cells from single cell (1cell) extraction or pooling of cells (3 cell or 5 cells). Spectra were obtained using iPLEX® Pro chemistry on the MassARRAY® System. Location of wild type (**WT**) and mutant allele (Mut). **A-C** Spectra of 1, 3 and 5 cell pools were from samples that underwent both whole genome amplification and the iPLEX® Pro assay. **D-F** Spectra of 1, 3 and 5 cell pools that did not go through whole genome amplification but were only processed using standard iPLEX® Pro PCR amplification.

Conclusions

MassARRAY® technology successfully detected mutations in single model CTCs that were individually picked from a blood sample processed by the AccuCyte – CyteFinder system both with and without whole genome amplification. Integrating CTC isolation with MassARRAY® technology may be a practical way to identify and monitor known cancer mutations non-invasively.

Significance

This is a simple and rapid way to identify multiple mutations in single cells, with the potential for analysis without introduction of sequence error by low fidelity whole genome amplification.