Next-generation sequencing of circulating tumor cells isolated from peripheral blood of patients with head and neck or gastrointestinal cancer

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Background

- Real –time monitoring of tumor biology provides crucial information for selecting the most appropriate therapy.
- Circulating tumor cells (CTCs) can reflect current tumor status at primary sites and metastases using blood samples without invasive procedures.
- Current CTC capture platforms employ flow cytometry [1], fluorescence and magnetic-activated cell sorting methods [2], gradient centrifugation [3], filtration [4], or droplets [5].
- The ClearCell FX system (Clearbridge Biomedics, Singapore) uses a label-free inertial microfluidics approach based on biomechanical properties, and is able to capture CTCs independent of their EpCAM expression.
- We previously reported that higher CTC counts were isolated by using ClearCell FX system than with the CellSerch system [6].

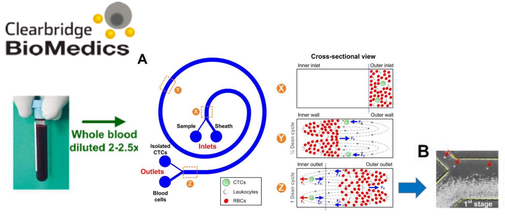
Objectives

- To investigate tumor biology by genomic profiling of CTCs using next-generation sequencing (NGS).

Eligibility criteria

(1) i) Histologically proven head and neck cancer patients, or
ii) Histologically proven, curatively unresectable, metastatic or recurrent gastrointestinal cancer patients
(2) Age: ≥ 20
(3) Written informed consent

Clear Cell FX system Mechanism of Isolation



- CTCs are enriched from blood components using Dean Flow Fractionation. A) Patient blood and sheath fluid are pumped in and are separated by a density gradient (X). The tube curvature results in shear and lift forces that cause cell migration across the density gradient (Y). Rate of movement is depends on cell size with smaller cells travelling faster. (Z) At 1 dean cycle the larger CTCs are most separated from the smaller blood cells and are drawn off. B) A representative image of point Z from whole blood spiked with tumor cells [7].

Methods

Isolation of CTCs

- 5.0-7.5 ml of blood from each patient were collected into EDTA-2Na tubes. CTCs were isolated using the ClearCell FX system™ as described previously [7].
- To count CTCs, we stained the isolated cells with anti-pan CK antibodies for epithelial cells and confirmed that CTCs were isolated.

Next generation sequence (NGS)

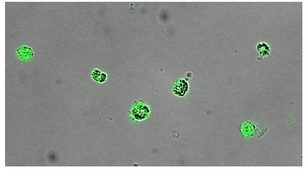
- Whole-genome amplification was performed using DNA extracted from CTCs.
- DNA from CTCs and genomic DNA from buffy coat were analyzed by NGS at National Cancer Center Research Institution.
- NGS was performed using the Ion Personal Genome Machine (PGM, LifeTechnologies, USA).

ABL1	EZH2	JAK3	PTEN
AKT1	FBXW7	IDH2	PTPN11
ALK	FGFR1	KDR	RB1
APC	FGFR2	KIT	RET
ATM	FGFR3	KRAS	SMAD4
BRAF	FLT3	MET	SMARCB1
CDH1	GNA11	MLH1	SMO
CDKN2A	GNAS	MPL	SRC
CSF1R	GNAQ	NOTCH1	STK11
CTNNB1	HNF1A	NPM1	TP53
EGFR	HRAS	NRAS	VHL
ERBB2	IDH1	PDGFRA	
ERBB4	JAK2	PIK3CA	

Ion Ampliseq Cancer Panel

- The Sequencing data were analyzed by Ion Reporter™ Software.
  - Established limit of detection is as follows;
  - SNV and Indel: ≥ 5% allele frequency with ≥ 250 reads

Detection of CTCs by immunofluorescence



Fluorescence images of sorted CTCs stained for cytokeratin (green)

Results

Patient characteristics

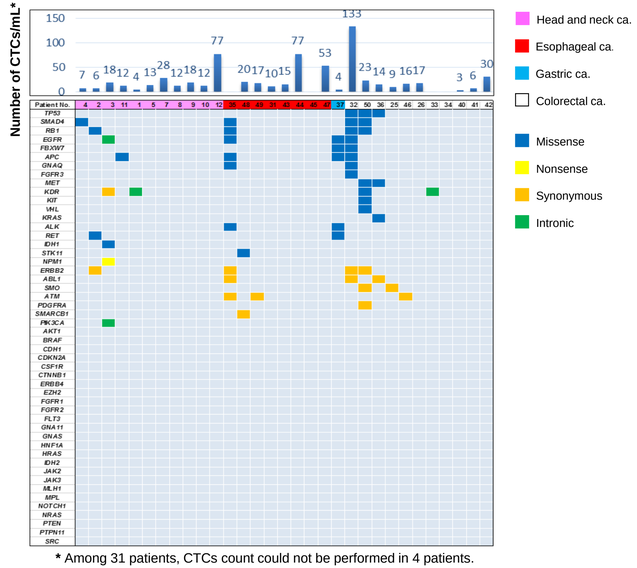
(A) head and neck cancer (n=11)

Clinical feature	Female	Male	Total
Age years: median (range)	73.5 (59-77)	67 (42-80)	72 (42-80)
Primary tumor site			
Oral cavity	3	2	5
Salivary gland	1	0	1
Pharynx	1	3	4
Cervical esophagus	1	0	1
Histology			
Squamous cell carcinoma	5	5	10
Adenoid cystic carcinoma	1	0	1
Stage (UICC TNM 7th)			
II	2	1	3
III	0	1	1
IV	4	3	7

(B) gastrointestinal cancer (n=20)

Clinical feature	Female	Male	Total
Age years: median (range)	61.5 (63-73)	59.5 (46-67)	61.5 (46-73)
Primary tumor site			
Esophagus	1	7	8
Stomach	1	0	1
Colon and Rectum	3	8	11
ECOG performance status at consent			
0	3	6	9
1	0	10	10
2	1	0	1
Disease status			
Stage IV	1	5	6
Recurrence	3	11	14
Number of prior chemotherapy lines			
0	1	8	9
1	1	2	3
2	1	4	5
≧4	1	2	3

Somatic mutations detected by target sequencing



* Among 31 patients, CTCs count could not be performed in 4 patients.

Conclusions

- We were able to effectively capture CTCs from patients with HN, EC, GC and CRC, and successfully perform NGS of CTCs using a microfluidic separation system without antibodies.
- The next trial is now ongoing to assess correlations between emergence of gene mutations in CTCs and changes in therapeutic effect during molecular-targeted therapy.

References

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