Circulating Tumor Cells in metastatic lung cancer enriched by EpCAM expression and physical characteristics

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Background

Circulating tumor cells (CTC) measured with the CellSearch system in patients with metastatic carcinomas are associated with poor survival. The frequency of CTC detected by the CellSearch system in non-small cell lung cancer (NSCLC) patients is relatively low, raising the question: are some CTC not detected by the CellSearch system?

To investigate this, a device was designed that collects the sample material of the individual samples that are discarded by CellSearch. This collected waste is filtered for CTC isolation based on physical characteristics and the CTC are stained with a cocktail of antibodies. Also, additional antibodies were added to the CellSearch system to broaden the coverage of cytokeratins and leukocytes.

Study design

Study design From each patient 7.5 ml is prepped by CellSearch and direct filtration. CellSearch waste is collected, filtered and separately stained.

Methods

The Autoprep Sample Collection Device (ASCD) uses optical sensing to detect the presence of blood in the waste tube of the CellTracks Autoprep. It collects the waste of individual samples in a 50 mL conical tube. After collection, the blood is passed with 100 mbar pressure through a 8x8 mm² microfabricated silicon microsieve containing 60,000 pores of 5 µm in diameter.

Figure 1 The Autoprep Sample Collection Device (ASCD) collects the waste from patient samples (A). A pump with disposable filtration unit filters the cells collected by the ASCD (B). The staining holder with microsieve slide is for staining and detection of CTC captured on the microsieve after filtration (C).

Cell lines

The performance of the ASCD and microsieve filtration was tested using four pre-stained EpCAM+ and EpCAM- cell lines: Colo320, T24, SW480 and SKBR3.

	Spike A "Big Cells"		Spike B "Small Cells"	
	T24 EpCAM -	SK-BR-3 EpCAM +	Colo-320 EpCAM -	SW480 EpCAM +
Healthy Donor (n=5)				
CellTracks	2% (±1)	87% (±12)	2% (±2)	91% (±13)
Sieve	59% (±9)	2% (±1)	18% (±6)	6% (±7)

Table 1 Cells are spiked in 7.5 mL of blood collected in CellSave tubes from healthy volunteers.

Staining

Image analysis

Figure 2 Image processing steps to determine the expression of extra markers. A threshold for DAPI and PE is determined for all images in a cartridge. Each event above the DAPI threshold is evaluated in all channels. If an overlapping PE Mask is found, this is used to measure additional markers.

The staining protocol and image analysis was tested with healthy donor samples.

#	Healthy control: not spiked		Healthy control: spiked	
	CellSearch	Filtration	CellSearch	Filtration
1.	0	0	0	80
2.	0	0	0	8
3.	0	0	0	5
4.	0	1	0	88
5.	0	0	0	21
6.	0	1	0	33

Table 2 Samples from healthy donors: not spiked and spiked with 10 to 100 cells of the EpCAM- non-small lung cancer cell line NCI-H1650.

Preliminary results

Patients with NSCLC (enrollment is ongoing) were processed on the CellSearch and filtration system between 24 and 96 hours of collection. The cells on the microsieves were stained with a nucleic acid dye and antibodies recognizing leukocytes and all cytokeratins. Additional antibodies were added to the CellSearch test to cover all cytokeratins and broaden the coverage of leukocytes.

Patient #	CellSearch			Whole blood Filtration	CellSearch Automated Analysis			
	CTC CellTracks	CTC Waste	Total CTC	CTC	CK PE+	CK PE+ CK FITC+	CK *FITC+	Total
1.	0	0	0	0	0	0	2	2
2.	0	0	0	0	0	0	1	1
3.	2	0	2	0	3	2	10	13
4.	0	0	0	0	0	0	11	11
5.	0	0	0	0	0	0	9	9
6.	11	0	11	NA	4	1	5	9
7.	0	NA	0	0	0	0	2	2
8.	2	0	2	2	2	1	1	3
9.	1	30	31	14	0	0	6	6
10.	29	14	43	11	38	13	3	41
11.	AB	29	29	3	NA	NA	NA	NA
12.	2	14	16	0	0	0	10	10
13.	0	6	6	38	0	0	1	1
14.	0	1	1	12	1	0	18	19
15.	0	3	3	3	1	0	9	10
16.	2	4	6	9	2	1	16	18
≥1 CTC (%)	38	50	69	50	44	25	94	94
≥10 CTC (%)	13	25	31	25	6	6	31	44

Table 3 Overview of CTC found in NSCLC patients. *Some CK-FITC+ events are due to debris which would be discarded in a manual count.

Figure 3 Thumbnail gallery showing a CTC and white blood cells after filtration on a sieve.

Conclusion

We combined the CellSearch system with a device for collecting and filtering the CellSearch waste. On cell lines this demonstrated that low EpCAM expression results in the presence of CTC in the waste, that otherwise would not be detected by the CellSearch system. In NSCLC additional CTC can be detected but it still remains to be determined whether these CTC – not detected by the original CellSearch approach – are also of clinical relevance.

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