Characterization of Circulating Tumor Cells Isolated from Bladder Cancer Patients Using ApoStream® Reveals Heterogeneity and Biomarkers of Epithelial-Mesenchymal Transition

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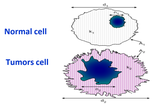
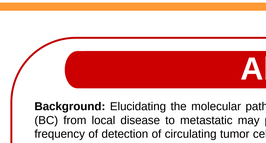
Abstract

Background: Elucidating the molecular pathways that drive the progression of metastatic bladder cancer (BC) from local disease to metastatic may provide insight into prognosis and potentially, treatment. The frequency of detection of circulating tumor cells (CTCs) in metastatic BC patients is only 30% using EpCAM based methods. Reports have shown that EpCAM based methods detect only a fraction of CTCs and miss the heterogeneous subpopulations of CTCs related to epithelial to mesenchymal transition (EMT). EMT is a hallmark of cellular invasion and metastasis and CTCs undergoing EMT may have prognostic value in BC if reliable detection and characterization methods were developed. Here we used ApoStream®, a novel antibody-free CTC isolation device, to isolate CTCs and perform molecular characterization. **Methods:** Blood samples from 13 early stage or metastatic BC patients were collected and processed using ApoStream®. Isolated cells were immunophenotyped using a multiplexed immunofluorescence assay for CK, CD45, DAPI, uroplakin, vimentin and Twist. Laser scanning cytometry analysis was applied to identify subsets of $CK^+CD45^-DAPI^+$ or $CK^-CD45^-DAPI^+$ cells for the expression and distribution of uroplakin, vimentin and Twist. Urovysion FISH analysis was performed on CTCs from 6 BC patients. **Results:** $CK^+CD45^-DAPI^+$ cells were detected in 4/13 (31%) of patients with vimentin detected in this subset. $CK^-CD45^-DAPI^+$ cells were detected in 8/13 (62%) of patients with Twist expression detected in this subset. Uroplakin expression was not detected. Chromosomal abnormalities were detected in $CK^-CD45^-DAPI^+$ cells isolated from the blood of 3/6 (50%) BC patients. **Conclusions:** ApoStream® isolated cells from the blood of BC patients with phenotypic and genotypic characteristics of CTCs. The $CK^{+/-}CD45^-DAPI^+$ Twist and vimentin phenotype indicates a population of circulating cells with relevant biomarkers of EMT and may represent an important population of CTCs mediating disease progression. Detection of chromosomal abnormalities on non-canonical CTCs highlights heterogeneity and underscores the need for expanded definitions of CTCs in BC.

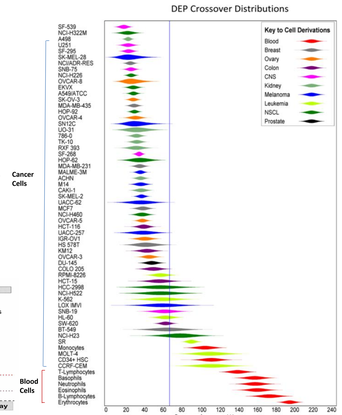
ApoStream® Technology



(A) Dielectric polarizability of cells are dependent upon cell diameter, membrane morphology and conductivity. Inherent differences in morphology of CTCs and normal cells result in different dielectric polarization charges when exposed to an AC electric current.



(B) Dielectrophoretic, hydrodynamic and sedimentation forces are utilized to attract CTCs and repel normal cells from the chamber floor. CTCs are collected through a port located in the chamber floor while normal cells flow into a waste port.



(C) Cross-over frequencies from different tumor cell types including breast, colon, ovarian, lung and melanoma cell lines and from peripheral blood mononuclear cells (PBMCs) were determined.[2] The differences in cross-over frequencies between cancer and normal cells enable ApoStream™ to separate CTCs from normal cells.

ApoStream® Prototype Device

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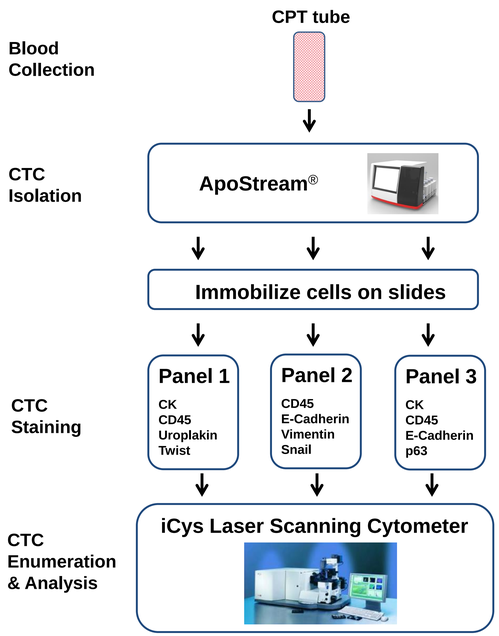
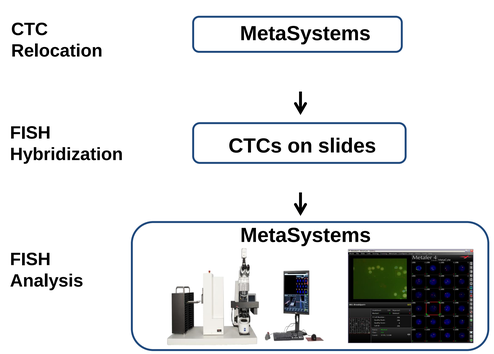
Study Design

CTC Analysis Schematic



FISH Analysis Schematic



Enumeration of CTC Phenotypes

Table 1.

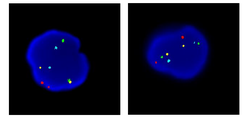
Patient Information		Enumeration of Cell Phenotypes Isolated by ApoStream®					
No.	Sponsor ID	CK^+CD45^-	CK^-CD45^-	Total $TWIST^+$ $DAPI^+$	$TWIST^+$ $CD45^+$ $DAPI^+$	$TWIST^+$ $CD45^-$ $DAPI^+$	Cell Genotypes by Urovysion® FISH Analysis
1	M12986	0	1	0	0	0	WT
2	M13017	0	1	2	2	0	Abnormal
3	M13005	0	2	305	302	3	WT
4	M13028	0	13	93	69	24	NA
5	M13029[a]	0	7	179	142	37	NA
6	M13032[a]	0	6	122	122	0	WT
7	M13047	0	0	43	43	0	WT
8	M13056[a]	0	1	1	1	0	Abnormal
9	M11532	0	15	91	89	2	WT
10	M13075[a]	1	0	324	304	4	Abnormal
11	M13088[a]	3	0	0	NA[b]	0	Abnormal
12	M13083[a]	4	0	0	NA[b]	0	WT
13	M13121[a]	5	0	0	NA[b]	0	WT

NA- not analyzed
[a]- denotes metastatic patient
[b]- TWIST staining not performed
WT-wild type

Urovysion® FISH Analysis

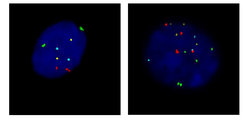
Patient ID	UroVysion FISH Genotypes on all Cells	Final Interpretation
M13121	2R:2G:2A:2Y (600 Cells)	WT

Red (R): CEP3
Green (G): CEP7
Aqua (A): CEP17
Gold (Y): 9p21(LSI)



Patient ID	UroVysion FISH Genotypes on all Cells	Final Interpretation
M13088 (Urine Sample)	4~5R:4~6G:3~4A:4Y (156 Cells) 2R:2G:2A:2Y (43 Cells)	Abnormal

Red (R): CEP3
Green (G): CEP7
Aqua (A): CEP17
Gold (Y): 9p21(LSI)



Patient ID	UroVysion FISH Genotypes on all Cells	Final Interpretation
M13088 (Blood Sample)	2R:2G:2A:2Y (597 Cells) 3R:2G:3A:3Y (1 Cell) 3R:3G:3A:3Y (1 Cell) 2R:2G:3A:2Y (1 Cell)	Abnormal

Red (R): CEP3
Green (G): CEP7
Aqua (A): CEP17
Gold (Y): 9p21(LSI)

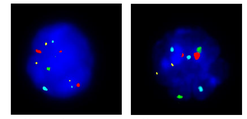


Figure 2. Representative images of UroVysion® FISH genotypes on two patient samples. Chromosomal abnormalities were found in the urine sample and ApoStream® enriched blood sample from patient M13088.

Biomarker Expression in CTCs

Table 2.

Patient Information		Cell Phenotypes Isolated by ApoStream®							
		CK^+ $CD45^-$			CK^- $CD45^-$			$Vimentin^+$ $CD45^-$	
No.	Sponsor ID	Count	MFI of Twist	MFI of Uroplakin	Count	MFI of Twist	MFI of Uroplakin	Count	MFI of Vimentin
1	M12986	0	0	0	1	101,833	0	N/A	N/A
2	M13017	0	0	0	1	124,806	0	N/A	N/A
3	M13005	0	0	0	2	219,142	0	N/A	N/A
4	M13028	0	0	0	13	680,463	0	N/A	N/A
5	M13029	0	0	0	7	746,614	0	N/A	N/A
6	M13032	0	0	0	6	164,901	0	N/A	N/A
7	M13047	0	0	0	0	0	0	N/A	N/A
8	M13056	0	0	0	1	204,163	0	N/A	N/A
9	M11532	0	0	0	15	313,469	0	N/A	N/A
10	M13075	1	-	0	0	N/A	N/A	N/A	N/A
11	M13088	3	115322	2784570	0	N/A	N/A	0	N/A
12	M13083	4	-	0	0	N/A	N/A	7	293980
13	M13121	5	-	454667	0	N/A	N/A	6	631006

*N/A denotes no CTCs detected in sample or staining was not performed; MFI = Mean Fluorescence Intensity.

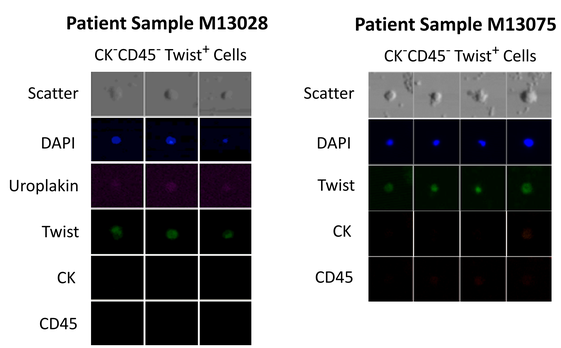


Figure 1. Representative images of the expression of Uroplakin and TWIST markers on the $CK^-/CD45^-$ cell population.

Summary

- ApoStream® isolated heterogeneous populations of CTCs from 13 bladder cancer patients.
  - 31% (4/13) patients with $CK^+CD45^-DAPI^+$ cells.
  - 62% (8/13) patients with CK^-CD45^-DAPI cells. Twist expression was detected in this subset.
- Urovysion® FISH testing on ApoStream® enriched blood samples was performed on 11 samples. Chromosomal abnormalities were detected in:
  - 3/6 (50%) metastatic bladder cancer patients.
  - 1/5 (20%) non-metastatic bladder cancer patients.
- Urovysion® FISH testing showed chromosomal abnormalities in a matched urine sample and ApoStream® enriched blood sample from patient M13088--thus confirming the functionality of the test for identifying CTCs independent of immunophenotyping.
- ApoStream® isolated a mixed population of CTCs with relevant biomarkers of EMT and provides an approach to characterize subpopulations of BC cells which may prognostic value.

References

1. Gupta, V., *et al*. ApoStream™, a new dielectrophoretic device for antibody independent isolation and recovery of viable cancer cells from blood. *Biomicrofluidics* 2012 6, 024133.
2. Sangjo Shim et al. Dielectrophoresis has broad applicability to marker-free isolation of tumor cells from blood by microfluidic systems. Biomicrofluidics, 7, 011808, 2013.