Single-cell low pass whole genome sequencing for copy-number profiling in circulating tumor cells from prostate, non-small cell lung and breast cancers

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BACKGROUND

Circulating tumor cells (CTCs) have been considered as an alternative to tissue biopsy for providing readouts of both germline-specific and tumor-derived genetic variants. Copy number variations (CNVs) play an important role in molding the genomes of cancers and have shown clinical value as prognostic signatures and for treatment selection. The current study aimed to evaluate CNVs at single-cell resolution and explore the potential clinical utility of tracking this key mechanism of cancer progression.

METHODS

- Blood samples were collected in Streck tubes from 40 stage II-IV cancer patients (5 prostate cancer, 15 non-small lung cancer (NSCLC), 20 breast cancer (BC)) before treatment.
- CTCs was identified as cells containing an intact nucleus, without CD45 expression, and with cytokeratin (CK) expression.
- Genomic analysis of chromosomal CNVs by low pass whole genome sequencing (Lp-WGS) and number of large-scale transitions (LSTs) calculation were performed in the CTCs.

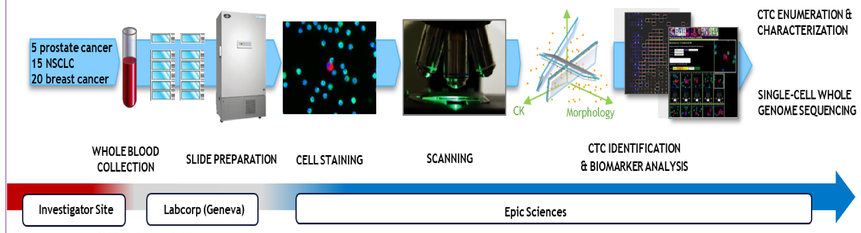
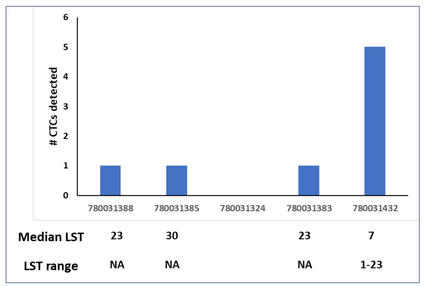


Figure 1. CTC Workflow

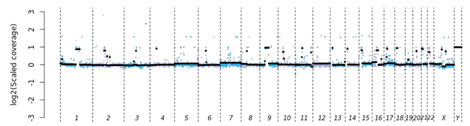
RESULTS – Prostate Cancer Samples



Selected genes with frequent CNVs detected

Gene	Gain	Loss	No Change
AR		8	
RB1		6	2
BRAF		6	2
ANKRD18B	6	1	1
CEP170	6		2
GAGE8	6		2
TP53	1	1	6
PTEN	1		7

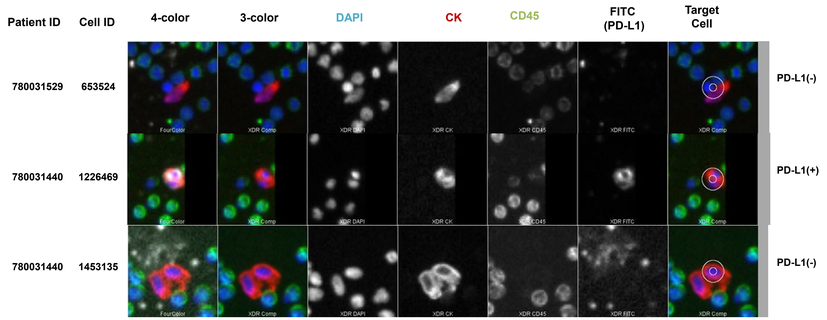
Example Lp-WGS CNV calling plots

Patient ID	LST Score for cell	CNV Plot	CTC Image*
780031385	23		

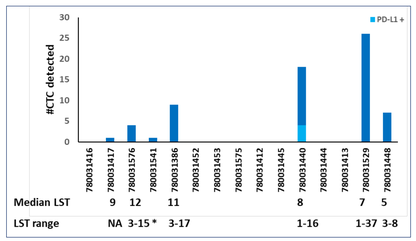
3-color overlay for DAPI, CK, CD45 as none AR N-term positives

RESULTS – NSCLC Samples

Representative images of CTCs identified



Slides were stained with DAPI, CK, CD45 and PD-L1. Individual CTCs were identified by Epic Sciences' algorithm and visually confirmed.
FITC: Fluorescein isothiocyanate

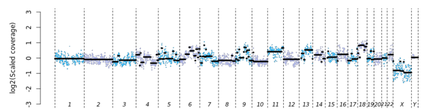
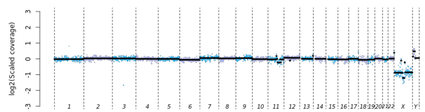
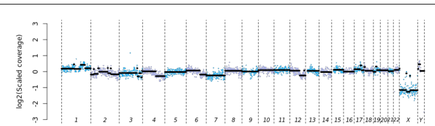


* CTC could not be picked for Lp-WGS

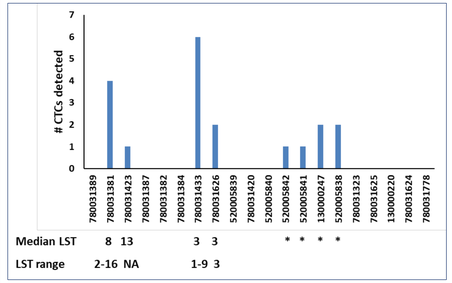
Selected genes with frequent CNVs detected

Gene	Gain	Loss	No Change
MED12	1	51	10
GAGE8	6	33	23
TP53	18	3	41
SPOP	18	9	35
RB1	10	3	49
AKT1	4	3	55
KRAS	8	8	46

Example Lp-WGS CNV calling plots

Patient ID	Cell ID	LST Score for cell	CNV Plot
780031385	30	653524	
780031440	6	1226469	
780031440	14	1453135	

RESULTS – Breast Cancer Samples

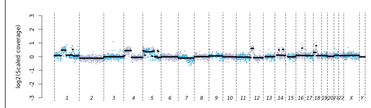
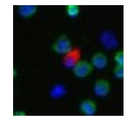


* CTC could not be picked for Lp-WGS

Selected genes with frequent CNVs detected

Gene	Gain	Loss	No Change
CXCR4		9	5
ATM		6	8
EGFR		4	10
KRAS	6	1	7
ERCC2	6	3	5
TP53	1	2	11
BRCA1	2	0	12
BRCA2	2	0	12

Example Lp-WGS CNV calling plots

Patient ID	LST Score for cell	CNV Plot	CTC Image
780031423	13		

3-color overlay for DAPI, CK, CD45

CONCLUSIONS

- Our data demonstrated that Lp-WGS of DNA from CTCs can detect CNVs loss and assess disease heterogeneity at the single-cell level.
- Since genome-wide CNV profiles can be used to characterize genomic instability, it is anticipated that the integrative analysis of morphology and genomics in CTCs can potentially complement tissue and/or ctDNA analysis to determine HRD, enabling in-depth investigation of tumor heterogeneity and individualized clinical assessment.

REFERENCES

1. Schonhoft JD, Zhao JL, Jendrisak A, Carbone EA, Barnett ES, Hullings MA, Gill A, Sutton R, Lee J, Dago AE, Landers M, Bakhoum SF, Wang Y, Gonen M, Dittamore R, Scher HI. Morphology-Predicted Large-Scale Transition Number in Circulating Tumor Cells Identifies a Chromosomal Instability Biomarker Associated with Poor Outcome in Castration-Resistant Prostate Cancer. Cancer Res. 2020 Nov 15;80(22):4892-4903. doi: 10.1158/0008-5472.CAN-20-1216.

ACKNOWLEDGEMENTS AND DISCLOSURES

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