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Comparative Single Cell Transcriptome Profiling of BL0293 PDX Primary Tumors, CTCs, and Metastatic Sites

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MOCHA

Abstract

Background: A PDX bladder cancer model, BL0293-F563, spontaneously metastasizes to the liver and bone, and sheds high numbers of circulating tumor cells (CTCs). This PDX model provides a unique opportunity to explore the relationships between primary tumors, CTCs, and metastases.

Methods: BL0293-F563 tumors (available from the NCI Patient-Derived Models Repository [https://pdmr.cancer.gov/] and originally developed by Jackson Laboratories) were implanted into NSG mice, and primary tumors, metastatic nodules in the liver, and blood were collected at maximal allowable tumor burden. Tumor tissue was dissociated using Miltenyi Tumor Dissociation Kit with OctoDissociator, and Human CTCs were enriched from mouse blood through negative selection with anti-mouse CD45 and anti-mouse MHC-1 magnetic beads. Single cell sequencing was done using 10X Genomics 3' gene expression assay v3.1. Data processing and analysis was done using 10X Genomics' Cell Ranger pipeline, Seurat, and cNMF.

Results: single cell RNAseq data from primary tumors, CTCs, and metastases from 9 mice were aggregated into a single dataset, and cells were classified into 22 clusters using Seurat FindNeighbors. All clusters contained cells from multiple sites (primary tumor, CTCs, metastases), but three clusters were enriched in CTCs and one cluster was composed of mostly primary tumor cells. All clusters exhibited epithelial-like gene expression signature scores, suggesting that CTC shedding was occurring without prominent epithelial-mesenchymal transition. CTC-enriched clusters showed elevated expression of RHO pathway genes, implicating ameboid-like migration in CTC shedding in this PDX model. Consistent with expected differences in oxygenation states, CTC-enriched clusters exhibited a lower hypoxia gene expression score than primary tumor and metastasis-enriched clusters. CTC-enriched clusters also showed higher expression of oxidative phosphorylation genes, suggesting metabolic differences between CTCs and cells from other sites. Additionally, two of three CTC-enriched clusters had elevated expression of mitosis-associated genes, indicating that at least some subpopulations of CTCs are actively cycling. A metastasis suppressor gene KISS1 was expressed in a subset of primary tumor cells but undetectable in CTCs, suggesting that KISS1 expression loss occurs before CTC shedding.

Conclusions: Utilizing single cell gene expression profiling, we have linked the gene expression profile of CTCs to specific cell subpopulations in primary tumors and metastases. We show that CTC-enriched cell clusters appear to maintain an epithelial phenotype. Subpopulations of CTC cells exhibit enrichment of motility-associated transcripts and features of active cell cycling. Our results implicate a known metastasis suppressor gene KISS1 in CTC shedding and metastatic dissemination in this PDX model.

Research Questions

- Are BL0293 CTCs closely related to any specific subpopulations of primary tumor cells and what are the transcriptional features of these subpopulations?
- Do BL0293 CTCs exhibit EMT or other notable transcriptional signatures?
- Do metastases revert to a primary tumor-like phenotype or do they retain and/or further evolve from a CTC-like transcriptome profile?

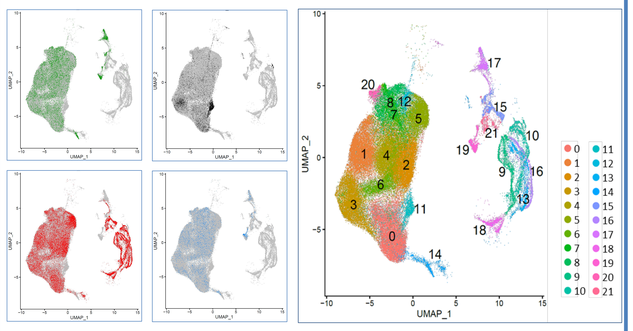
Results

Data summary

- PDX model: BL0293-F563 (bladder cancer)
- *In vivo:*
  - PDX tumors, were grown subcutaneously in NSG mice
  - Primary tumors, metastases and blood collected when primary tumors reached 2000 mm^3
- Samples:
  - Primary tumors: n=9
  - CTC: n=9
  - Liver metastases:
    - Individual nodules: n=13
    - Multiple nodules per sample: n=4
  - Lymph node metastases: n=3
- Sample preparation:
  - Primary tumors and metastases: Miltenyi Octo Dissociator and tumor dissociation kit
  - CTC enrichment: depletion of mouse cells using antibodies against mouse CD45 and MHC I
- Sequenced cells: n=136,048
  - Primary tumors: n=38,505
  - CTC: n=11,049
  - Liver metastases: n=48,175
  - Lymph node metastases: n=11,066
- Single cell sequencing:
  - 10X Genomics Chromium, 3' RNA assay v 3.1
  - cells per sample: 500 – 10,848
  - sequencing depth: >66,000 reads per cell

CTCs are associated with specific subpopulations of primary tumor cells

Clustering: FindNeighbors (Seurat)

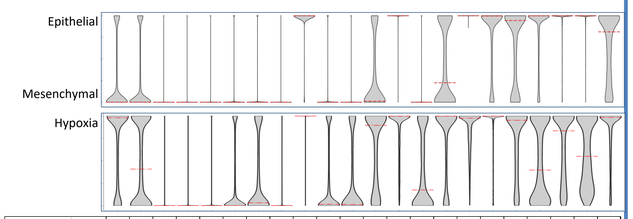


- primary tumors
- CTC
- liver metastases
- lymph node metastases

Sample type representation in each cluster: odds ratio
(Sample type fraction in cluster / sample type fraction in entire dataset)

		primary tumor	CTC	liver metastasis	LN metastasis
	cell count	38505	11049	48175	11066
mixed	0	14596	1.11	1.57	1.29	1.25
mixed	1	13425	1.45	1.35	1.04	1.38
mixed	2	12032	1.39	0.94	1.15	1.51
CTC-enriched	3	11497	1.13	2.21	1.17	1.06
mixed	4	9874	1.66	0.91	0.98	1.33
CTC-low	5	9324	1.37	0.61	1.30	1.26
mixed	6	5954	1.62	0.88	1.00	1.46
CTC-low	7	5571	1.71	0.57	0.99	1.45
	8	3850	1.99	0.63	0.69	1.73
metastasis-enriched	9	2796	0.01	0.14	2.78	0.00
metastasis-enriched	10	2485	0.03	0.54	2.68	0.02
CTC-enriched	11	2425	0.12	9.26	0.58	0.14
mixed	12	2004	1.69	2.00	0.65	1.58
metastasis-enriched	13	1826	0.01	0.09	2.79	0.02
mixed	14	1819	1.34	1.44	0.96	2.01
metastasis-enriched	15	1756	0.63	0.01	2.12	0.85
metastasis-enriched	16	1603	0.02	0.03	2.80	0.01
	17	1547	2.04	0.53	0.57	2.21
metastasis-enriched	18	1294	0.00	0.00	2.82	0.00
	19	1200	2.10	0.00	0.01	4.94
metastasis-enriched	20	1009	1.19	1.10	1.14	2.08
metastasis-enriched	21	908	0.58	0.00	2.26	0.43

CTCs exhibit diverse epithelial-mesenchymal and hypoxia gene expression profiles



cluster ID	7	5	4	1	2	3	8	6	17	0	12	20	15	11	14	10	19	21	13	9	16	18
cell count	5571	9324	9874	13425	12032	11497	3850	5954	1547	14596	2004	1009	1756	2425	1819	2485	1200	908	1826	2796	1603	1294
primary tumor 38505	1.71	1.37	1.66	1.45	1.39	1.13	1.99	1.62	2.04	1.11	1.69	1.19	0.63	0.12	1.34	0.63	2.10	0.58	0.01	0.01	0.02	0.00
CTC 11049	0.57	0.61	0.91	1.35	0.94	2.21	0.63	0.88	0.53	1.57	2.00	1.10	0.01	9.26	1.44	0.54	0.00	0.00	0.09	0.14	0.03	0.00
liver metastasis 48175	0.99	1.30	0.98	1.04	1.15	1.17	0.69	1.00	0.57	1.29	0.65	1.14	2.12	0.58	0.96	2.68	0.01	2.26	2.79	2.78	2.80	2.82
LN metastasis 11066	1.45	1.26	1.33	1.38	1.51	1.06	1.73	1.46	2.21	1.25	1.58	2.08	0.85	0.14	2.01	0.02	4.94	0.43	0.02	0.00	0.01	0.00
	CTC-low	CTC-low	mixed	mixed	mixed	CTC-enriched	mixed	mixed	mixed	metastasis-enriched	metastasis-enriched	CTC-enriched	mixed	metastasis-enriched	metastasis-enriched	metastasis-enriched	metastasis-enriched	metastasis-enriched	metastasis-enriched	metastasis-enriched	metastasis-enriched	metastasis-enriched

- CTC-associated clusters show a mix of epithelial-mesenchymal gene expression scores
- CTC-associated clusters tend to have a lower hypoxia signature than primary tumor or metastasis-enriched clusters

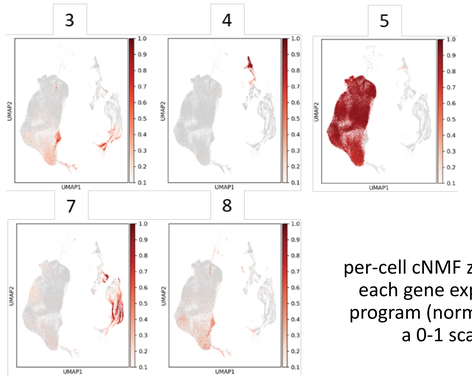
HALLMARK Epithelial-Mesenchymal Transition (mSigDb)
HALLMARK Hypoxia (mSigDb)

VAM: Frost 2020

8 distinct gene expression programs defined by cNMF analysis

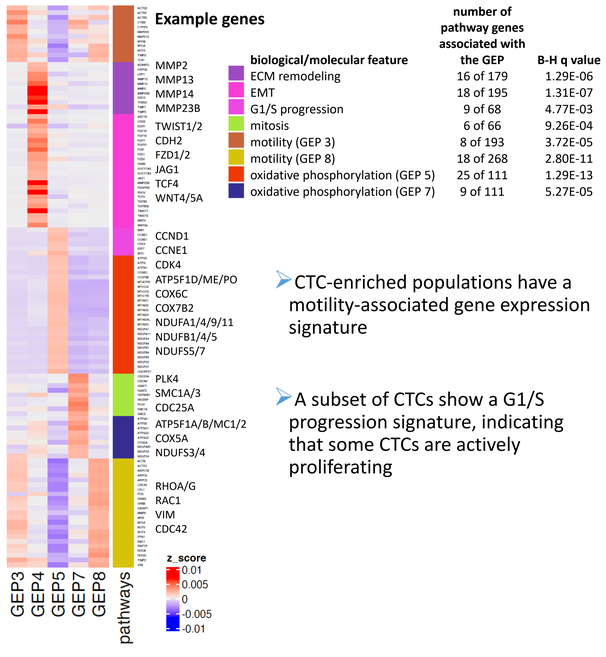
- Primary tumor-enriched: GEP 4, 5
- CTC-enriched: GEP 8
- CTC and metastasis enriched: GEP 3

	primary tumors	CTC	liver metastases	LN metastases
cell count:	37414	32801	55643	10190
% of all cells:	27.5%	24.1%	40.9%	7.5%
GEP				
1	0.55	0.14	1.82	0.97
2	0.05	0.16	2.32	0.00
3	0.03	1.32	1.64	0.04
4	2.20	0.19	0.46	2.17
5	1.42	0.46	0.96	1.40
6	0.40	0.64	1.76	0.23
7	0.02	0.18	2.32	0.02
8	0.01	3.99	0.08	0.01



per-cell cNMF z-score for each gene expression program (normalized to a 0-1 scale)

CTC-associated gene expression programs are signatures of oxidative phosphorylation, G1/S progression and motility

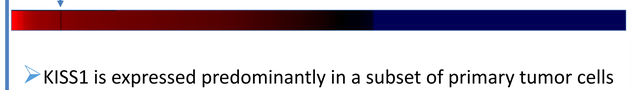


biological/molecular feature	number of pathway genes associated with the GEP	B-H q value
ECM remodeling	16 of 179	1.29E-06
EMT	18 of 195	1.31E-07
G1/S progression	9 of 68	4.77E-03
mitosis	6 of 66	9.26E-04
motility (GEP 3)	8 of 193	3.72E-05
motility (GEP 8)	18 of 268	2.80E-11
oxidative phosphorylation (GEP 5)	25 of 111	1.29E-13
oxidative phosphorylation (GEP 7)	9 of 111	5.27E-05

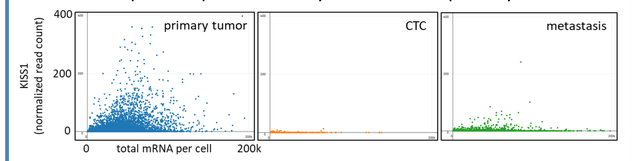
- CTC-enriched populations have a motility-associated gene expression signature
- A subset of CTCs show a G1/S progression signature, indicating that some CTCs are actively proliferating

KISS1 is a metastasis suppressor that is specifically expressed in BL0293 primary tumor cells

- BL0293 is at the high end of KISS1 expression distribution among PDMR models (bulk RNAseq of primary tumors)



- KISS1 is expressed predominantly in a subset of primary tumor cells



- BL0293 primary tumors contain KISS1-high and KISS1-low cells – this suggests that KISS1 expression loss occurs before CTC shedding
- Origination of CTCs from KISS1-low primary tumor cells may explain the high rate of metastasis in a model with high bulk expression of KISS1

Conclusions

- We identified subsets of CTCs with close relationship to primary tumor
- Subpopulations of CTCs appear to maintain an epithelial phenotype without EMT
- Subpopulations of CTCs show higher expression of mitosis associated genes, suggesting that some CTCs are actively progressing through the cell cycle

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Acknowledgements

Brandon Miller and Sonny Khin, PADIS

NCI-Frederick is accredited by AAALAC International and follows the Public Health Service Policy for the Care and Use of Laboratory Animals. Animal care was provided in accordance with the procedures outlined in the "Guide for Care and Use of Laboratory Animals" (National Research Council; 2011; National Academy Press; Washington, D.C.)

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.