FPN 28 P

MOLECULAR ANALYSIS FOR PRECISION ONCOLOGY VIRTUAL CONGRESS MAP

Validation of Cytokeratin Protein Expression in Epithelial Cell Lines and in Circulating Tumor Cells

Nirmal Raut[1*], Narendra Kale[2], Alain D'Souza[2], Pratim Chakraborty[2], Kumar Prabhash[3], Pankaj Chaturvedi[3], Jayant Khandare[2,4]

1. Bhaktivedanta Hospital and Research Institute, INDIA | 2. Actorius Innovations and Research Pvt. Ltd., Pune, Maharashtra, INDIA, 3. Tata Memorial Hospital, Mumbai, INDIA | 4. Actorius Innovations and Research Co., Simi Valley, CA, USA. Email: drnirmalraut@gmail.com

INTRODUCTION

The presence of **C**irculating **T**umor **C**ells (CTCs) in peripheral blood implicate metastatic progression, minimal residual disease (MRD), and predict overall survival in several epithelial cancers, including breast, prostate colorectal, and so on.

Cytokeratin (CK) 18 expression is a phenotypic trait of epithelial-mesenchymal transition (EMT). Its role in multiple drug resistance (MDR) and metastasis remains unknown.

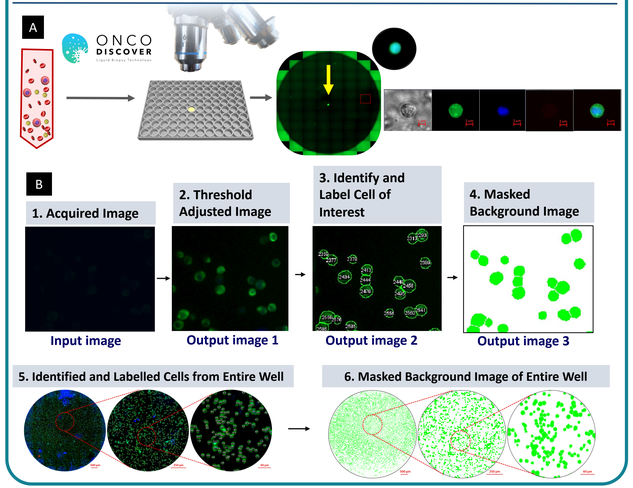
METHODS

We analyzed CK18 variance in epithelial cell lines (e.g. A549^{+}, MCF-7^{+}, and MEF^{-}, total n=192269) and CTCs (n=63) from different cancer phenotypes.

Followed by immunostaining, the fluorescence intensity was measured using Zeiss microscope, computer-assisted scanning and by customized *ImageJ*-macro tool (A-B 1-6).

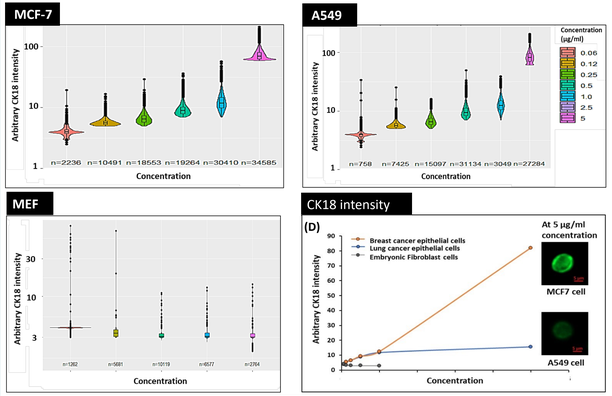
CK18 intensity (Anti-CK18 Ab conc: 0.06-6 μg/ml) and the binding constants (K_b) were measured using cell lines namely A549^{+}, MCF-7^{+} and MEF^{-}.

CTCs were isolated and enumerated from Head and Neck Squamous Cell Carcinoma (HNSCC) patients (CTRI/2018/03/012905) and clinical samples (e.g. breast, lung, colorectal, ovarian) using regulatory approved OncoDiscover® CTC detection platform (approved by DCGI, India).



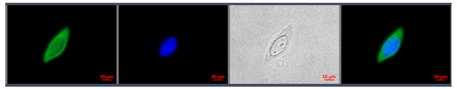
RESULTS

Comparative CK18 Intensities for MCF7, A549, and MEF cells

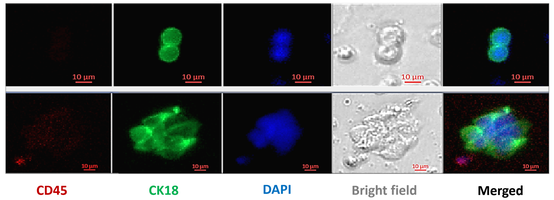


Violin and box plot depict comparative CK18 expression for MCF-7, A549, MEF. Distribution of MCF-7 and A549 cells showed higher CK18 intensity with increase in anti-CK18 antibody concentration. On the other hand, MEF cells didn't show CK18 expression. **(D)** Line plot showing the mean CK18 intensity increase for each population of cells with increase in anti-CK18 antibody concentration. *Inset:-* Labelled A549 and MCF-7 cells stained with different concentration of anti-CK18 antibody.

CK 18 Expression and Intensity in Cell Line



Circulating Tumor Cells and CTC Cluster



Cell, CTC and CTC cluster obtained from an Ovarian Cancer patient, immuno-stained with anti-CK18, CD45 and DAPI.

Change in CK18 intensity for MCF-7 and A549 cells, stored at 0^0 to 8^0 C and RT for 48hours

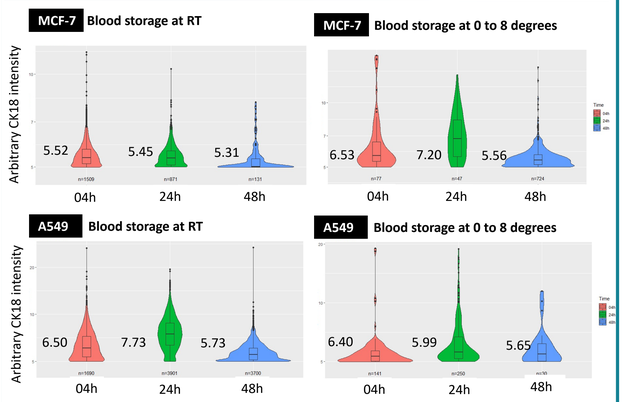
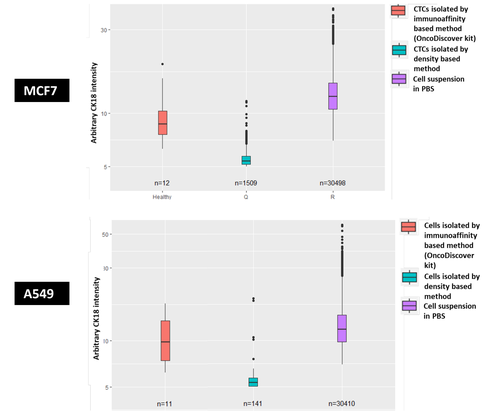


Table 1:
CK18 intensity for cell lines and CTC's derived from different tumor types

Sr. No.	Cell type/Cancer subtype		No. of Cells	Mean CK18 Intensity	Normalized Intensity	% change in CK18 intensity compared to MEF
1	MCF-7 (Breast)		30,498	12.49	4.1	310
2	A549 (Lung)		30,410	11.85	3.9	290
3	MEF (Fibroblast)		2,764	3.01	1.0	0
	CTC in Primary Cancer Types					
1	Breast		23	8.54	2.83	183
2	Lung		2	5.30	1.76	76
3	HNSCC	Tongue	15	5.60	1.86	86
4	HNSCC	Buccal	4	5.05	1.67	67
5	Colorectal		10	12.6	4.20	320
6	Ovarian		9	9.54	3.15	215

Fluorescence intensity was accounted in MEF (Fibroblast) cell line as a negative control for CK expression

CK18 intensity for MCF-7 and A549 cells, spiked in blood using OncoDiscover® CTC isolation Platform



RESULTS

- **CK18 mapping revealed diverse fluorescence intensities distribution in 03 cell lines, as well as in HNSCC, lung, breast, ovarian, and colorectal cancer CTCs (Table 1).**
- **The protein binding assay showed K_b 8.65 x 10^3 (M^{-1}) for MCF-7 and 7.9 x 10^3 for A549 cells, indicating concentration-dependent binding for CK18 expressing proteins on cells and may be varied in CTCs of different cancer types. This was compared to CK^{-} cell line (MEF).**
- **The normalized CK18 intensity was higher by 290% and 310% in MCF-7 (breast) and A549 (lung) cells, respectively, demonstrating the variation in CK18 expression.**
- **CTCs showed significant diversity in CK 18 expression in buccal mucosa, revealing the lowest expression(67%), while CTCs of CRC cancer type demonstrated the highest expression (320 %).**

CONCLUSIONS

- CKs are highly validated and implicated tumor markers in cancer diagnostics.
- Non-regulated CTC detection platforms pre-requisite the critical validations to eliminate the non-specificity of CTC count, which are highly imperative to clinical decisions in cancer management.

REFERENCES

1) Aceto et al, Cell 2014; 158(5): 1110 -1122, 2) American Society of Clinical Oncology (ASCO) – Journal of Clinical Oncology 2019,37(15) suppl, e14516, 3) American Association for Cancer Research (AACR) – Advances in Liquid Biopsy, Abstract No. A-35259260, 4) Pantel et al, Nat Rev Clin Oncol 2019;16(7):409-424, 5) CDSCO India License No. MFG/IVD/2019/000031.

CONTACT

Dr. Jayant Khandare

Actorius Innovations and Research, India, Actorius Co, USA. | www.oncodiscover.com

jayant@actorius.co.in | +91 9920526627