Patient-derived breast and melanoma circulating tumor cell (CTC) in vitro models as encouraging new tools for cancer research

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Poster 1087

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Abstract

Circulating Tumor Cells (CTCs) have emerged as powerful tools for understanding the mechanisms behind cancer biology, particularly in context of metastatic disease. Although rare, CTCs are increasingly recognized for their potential in the early diagnosis, monitoring, and progression of tumors. These cells can detach from a primary cancer tumor and enter the bloodstream, facilitating metastasis at distant tissue sites. As research into CTCs advances, new opportunities will continue to emerge to better manage therapeutic targets, especially for metastatic disease. Despite progress in CTC isolation techniques, detecting CTCs remains challenging due to their rarity in the bloodstream. Furthermore, long-term and scalable in vitro culture of CTCs has not been previously achieved. As a result, there is a significant lack of widely available CTC models for studying cancer biology, metastatic progression and development of new treatment strategies. ATCC is actively collaborating with various institutions to make CTCs widely available for cancer research, focusing on the development of standardized expansion and characterization protocols for CTCs isolated from clinical patient samples. In this study, we present the successful propagation and characterization of six well-established breast and melanoma CTC models originating from metastatic breast and melanoma disease: Brx50 (ATCC® CRL-3648™), Brx61 (ATCC® CRL-3649™), Brx142 (ATCC® CRL-3650™), MEL167 (ATCC® CRL-3651™), MEL182 (ATCC® CRL-3652™), and PEM78 (ATCC® CRL-3653™). We evaluated the genomic, proteomic, and functional characteristics of both the breast cancer and melanoma CTC models. Genetic profiling via sequencing revealed key oncogenic drivers potentially linked to metastatic behavior. Gene expression profiles were compared with commonly used breast and melanoma cell lines to highlight distinct molecular signatures. Immunofluorescence staining was performed to assess the distinct presence of breast cancer or melanoma molecular biomarker panels. Drug response assays were conducted to evaluate the sensitivity of the breast models to estrogen receptor (ER) inhibitors and melanoma models to BRAF inhibitors. Additionally, drug response profiles of the breast CTC models were compared to those of commonly used triple-positive and triple-negative breast cancer cell lines, while melanoma CTC models were compared to the A375 melanoma cell line and CRISPR-engineered drug-resistant variants. In conclusion, metastatic disease remains difficult to treat due to reduced therapeutic response rates and increased disease relapse potential. The six new widely available CTC lines—Brx50, Brx61, Brx142, MEL167, MEL182, and PEM78—represent robust and versatile models for investigating the role of CTCs in pre-clinical diagnostics, disease monitoring, and the progression of metastatic breast cancer and melanoma.

Background

- Circulating Tumor Cells (CTCs) are rare population of cells are released from a primary tumor site and circulate though the bloodstream enabling metastasis at secondary tissue sites.
- Utilizing the CTC iChip technique developed by Massachusetts General Hospital, Brx50, Brx61, Brx142, MEL167, MEL182, and PEM78 all were isolated from the blood samples of patients undergoing treatment for either breast or melanoma cancer.
- CTC cells represent versatile models for investigating new therapeutic targets and understanding tumor progression and metastasis.

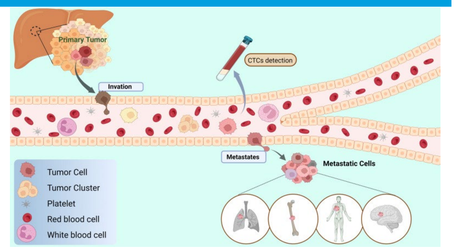

Diagram adapted from Hsu, C., et al. Journal of Clinical and Translational Hepatology, 12(12), 1020, 2024.

Results

Table 1: ATCC's available models for circulating tumor cells (CTC)

Cell Line	ATCC® Item Number	Disease Model	Gender	Age	Mutations
Brx50	CRL-3648™	Breast	Female	42	ER+/PR+/HER2-
Brx61	CRL-3649™	Breast	Female	54	ER+/PR+/HER2-
Brx142	CRL-3650™	Breast	Female	62	ER+/PR-/HER2-
MEL167	CRL-3651™	Melanoma	Male	49	BRAF (V600E), CDKN2A (R29*), STK11 (C418W)
MEL182	CRL-3652™	Melanoma	Female	37	BRAF (G464V), NRAS (Q61K), PDGFRA (E386K)
PEM78	CRL-3653™	Melanoma	Female	50	GNA11 (Q209L)

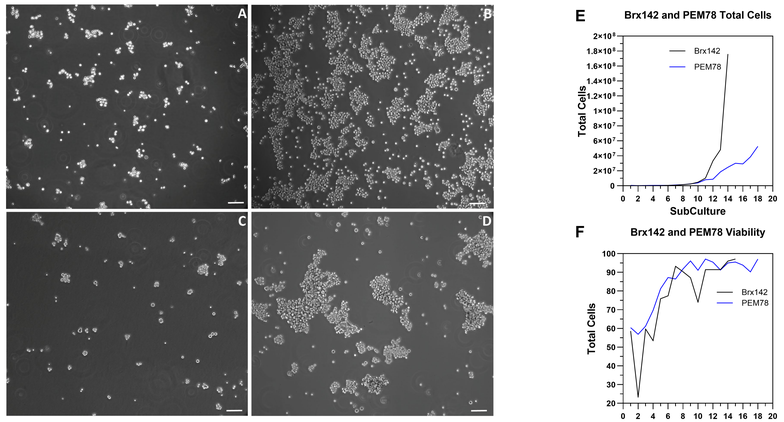

Figure 1: Morphology images and growth curves of Brx142 (ATCC® CRL-3650™) and PEM78 (ATCC® CRL-3653™) CTCs. (A) Brx142 low density. (B) Brx142 high density. (C) PEM78 low density. (D) PEM78 high density. Both cell lines each grow into medium sized clusters. Scale bars are 100 µm. (E) Total cell growth and (F) Cell viability of Brx142 and PEM 78 CTCs.

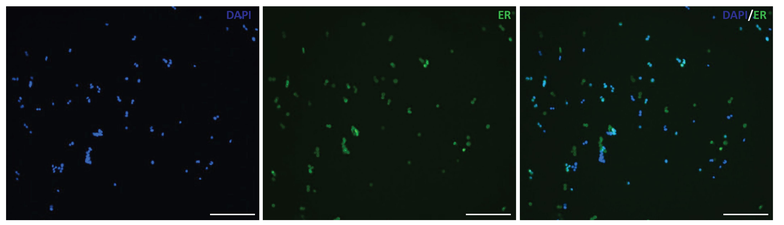

Figure 2: Immunofluorescent staining of Brx50 with a common breast cancer marker, Estrogen Receptor (ER). Representative images of Brx50 (ATCC® CRL-3648™) stained with breast marker ER (green) and DAPI nuclear stain (blue). Scale bar is 50 µm, images were acquired via digital camera (Zyla, Andor).

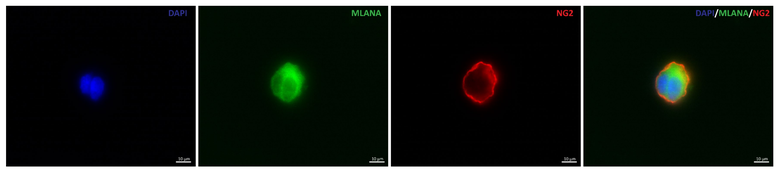

Figure 3: Immunofluorescent staining of PEM 78 with common melanoma markers MLANA and NG2. Representative images of PEM 78 CTC cells (ATCC® CRL-3653™) co-stained with melanoma markers MLANA (green), NG2 (red), and DAPI nuclear stain (blue). Scale bar is 10 µm, images were acquired using the Leica Mica confocal microscope.

References

- Hong, X., et al. Cancer Discovery, 11(3), 678-695, 2021.
- Yu, M., Bardia, et al. science, 345(6193), 216-220, 2014.
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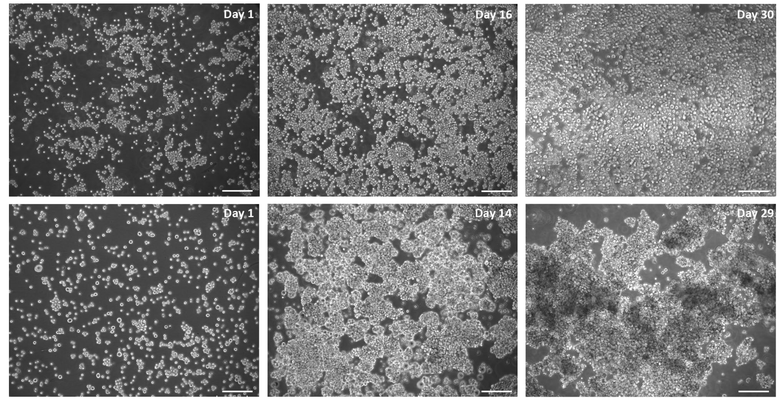
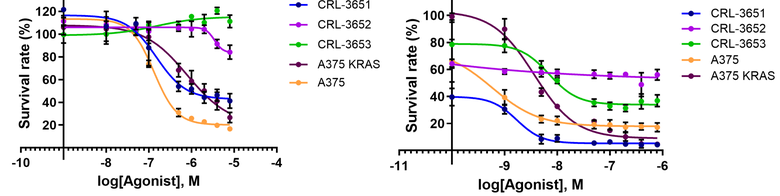


Figure 4: Long-term characterization of breast and melanoma CTCs. Cells were grown for 4 weeks resulting in larger cell clusters. Brx142 (Top) plated at $2x10^6$ and PEM78 (Bottom) plated at $1x10^6$ cells/ml were imaged throughout growth period. Scale bar is 100 µm.



	MEL167	MEL182	PEM78	A375	A375 KRAS
IC50	1.509e-007	3.465e-006	1.519e-007	1.325e-007	7.997e-007

	MEL167	MEL182	PEM78	A375	A375 KRAS
IC50	1.713e-009	7.996e-021	7.030e-009	5.818e-010	3.671e-009

Figure 5: Dose-response curve of melanoma CTCs 4-day treatment with the (A) BRAF inhibitor Vemurafenib or (B) MEK inhibitor Trametinib. MEL167 (blue line) showed drug sensitivity to Vemurafenib and Trametinib, MEL182 (purple line) showed slight drug sensitivity to Vemurafenib and resistance to Trametinib, and PEM78 (green) showed drug resistance to Vemurafenib and sensitivity to Trametinib as compared to the common melanoma model A375 (ATCC® CRL-1619™) (orange) and KRAS mutant-A375 isogenic line (ATCC® CRL-1619IG-1™) (purple). Cell viability was detected for each well using the CellTiter-Glo assay (Promega). Data represent mean ± SD; n=4).

Conclusions

- Brx50 (ATCC® CRL-3648™), Brx61 (ATCC® CRL-3649™), Brx142 (ATCC® CRL-3650™), MEL167 (ATCC® CRL-3651™), MEL182 (ATCC® CRL-3652™), and PEM78 (ATCC® CRL-3653™) are the first widely commercially available circulating tumor cell lines.
- The common breast marker ER is present on the surface (Figure 2) of Brx50 (ATCC® CRL-3648™) CTCs, and common melanoma markers MLANA and NG2 are present on the surface (Figure 3) of PEM78 (ATCC® CRL-3653™) CTC cells.
- The drug response to both the BRAF inhibitor (Vemurafenib) (Figure 5A) and MEK inhibitor (Trametinib) (Figure 5 B), two approved drug treatments for melanoma, showed varying drug responses between all 3 melanoma CTC models as compared to the common melanoma model A375 (ATCC® CRL-1619™) and the KRAS mutant-A375 isogenic line (ATCC® CRL-1619IG-1™).

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