New Emerging Patient-derived Circulating Tumor Cells (CTCs) as Promising Tools for Cancer Research

Abstract #1935

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Abstract

Circulating tumor cells (CTCs) play a significant role in understanding the mechanisms of cancer biology and, more specifically, metastatic tumors. CTCs are cells from a primary tumor that break away to circulate through the bloodstream and metastasize in other areas of the body. CTCs have been proposed as valuable tools for the early diagnosis, monitoring, and identification of tumor progression as they provide possible new therapeutic targets. However, CTCs have relatively low numbers in the bloodstream as compared to other cell types, which makes isolating and establishing a CTC line difficult. Further, while researchers are able to identify and isolate these cells with new techniques, there is a lack of established CTC lines that are widely available to the research community for basic mechanism studies and translational research. Collaborating with leading research institutions, ATCC® is putting effort into developing methods and protocols for the expansion and characterization of human CTC lines isolated from clinical patient samples. In this study, we demonstrated the propagation and characterization of a melanoma CTC line MEL 167 (ATCC® CRL-3651™), which originated from a metastatic melanoma patient sample. Here, we evaluated the MEL 167 cell line at the genetic and protein levels and assessed its bio-functional aspects. Genetic profiling of MEL 167 by sequencing analysis revealed various oncogenes that may enable the progression of metastasis. Immunofluorescence staining was performed to assess the presence of a distinct melanoma molecular marker panel. Additionally, the drug response to BRAF-inhibitors of MEL 167 was evaluated. We also compared the drug profile of this new CTC model to the established melanoma cell line model A375 (ATCC® CRL-1619™) and one other drug-resistant melanoma cell line, isogenic KRAS mutant-A375 Isogenic line (ATCC® CRL-1619IG-1™) that was created through CRISPR gene editing. We found that melanoma metastases are highly malignant with a low response rate to immunotherapies and high potential for relapse from drug-resistance stemming from tumor heterogeneity. As a newly emerging CTC model, MEL 167 (ATCC® CRL-3651™) offers a promising tool for pre-clinical studies and for evaluating CTCs in the early diagnosis and monitoring of metastatic melanoma.

Background

- Circulating tumor cells are a rare population of cells released from a primary tumor and circulate within the bloodstream allowing for metastasis at a secondary site.
- MEL 167 CTC cells were isolated from a patient's blood sample using the CTC-iChip technique developed by Massachusetts General Hospital.
- CTCs may hold potential for new therapeutic targets, monitoring and identification of tumor progression.

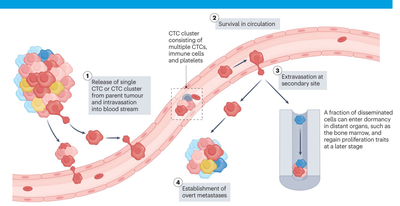


Diagram adapted from Nat Rev Clin Oncol 20(7): 487-500, 2023.

Results

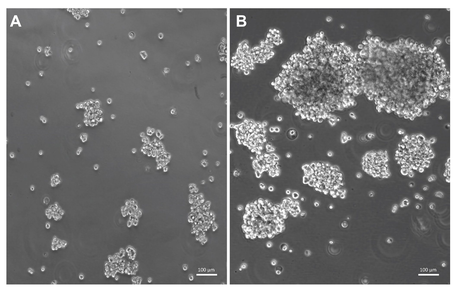


Figure 1: Melanoma Circulating Tumor Cell (CTCs) MEL167 (ATCC® CRL-3651™) morphology. (A) MEL 167 at low-density and (B) high density. Cells grow in small to medium sized clusters. Scale bars are 100 µm.

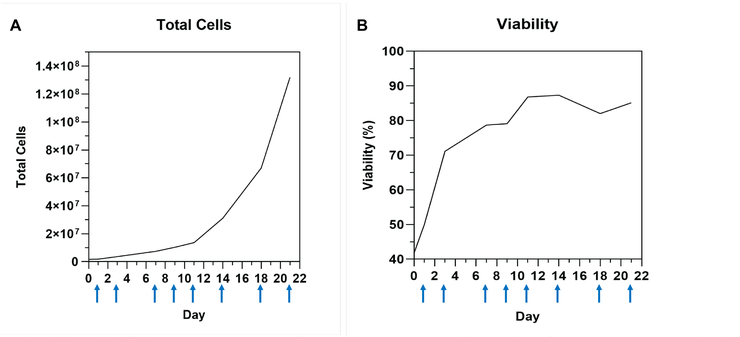


Figure 2: MEL 167 CTC growth curve. (A) Total cell number and (B) viability. Cells were plated at $2x10^5$ cells/mL and monitored over 21 days. MEL 167 cells require a complete medium change following 3-4 days of growth (blue arrows represent a sub-culture). Viability (B) shows the cells increasing to 75% by day 7 with continuous viability ≥ 80% by day 10.

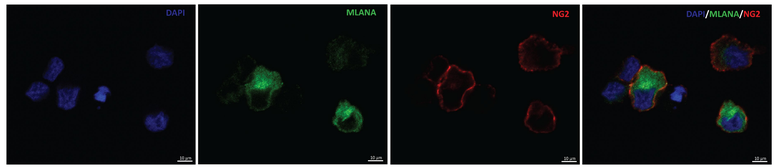
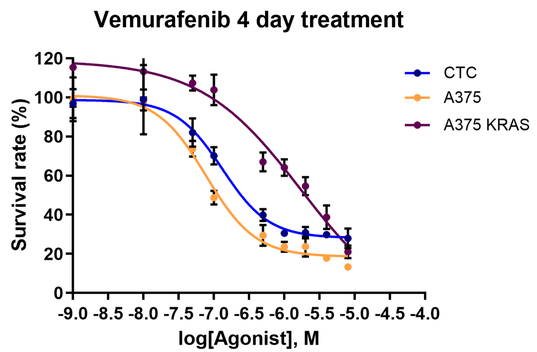


Figure 3: Immunofluorescent staining of MEL 167 with common melanoma markers MLANA and NG2. Representative images of MEL 167 CTC cells (ATCC® CRL-3651™) co-stained with melanoma markers MLANA (green) and NG2 (red), along with DAPI nuclear stain (blue). Scale bar is 10 µm, images were acquired using the Leica Mica confocal microscope.



	CTC	A375	A375 KRAS
IC50	1.322e-007	7.792e-008	1.728e-006

Figure 4: Dose response curve of MEL 167 CTC cells treated with the BRAF inhibitor, Vemurafenib. MEL 167 (ATCC® CRL-3651™) cells illustrate drug sensitivity to the BRAF inhibitor, vemurafenib at 132 nM (blue curve). CTC cells provide a similar drug profile as the common melanoma model, A375 (ATCC® CRL-1619™) (orange curve) with a higher relative drug sensitivity to vemurafenib as the KRAS mutant-A375 isogenic line (ATCC® CRL-1619IG-1™) (purple curve). Cell viability was detected for each well using the CellTiter-Glo assay (Promega). Data represent mean ± SD; n=4.

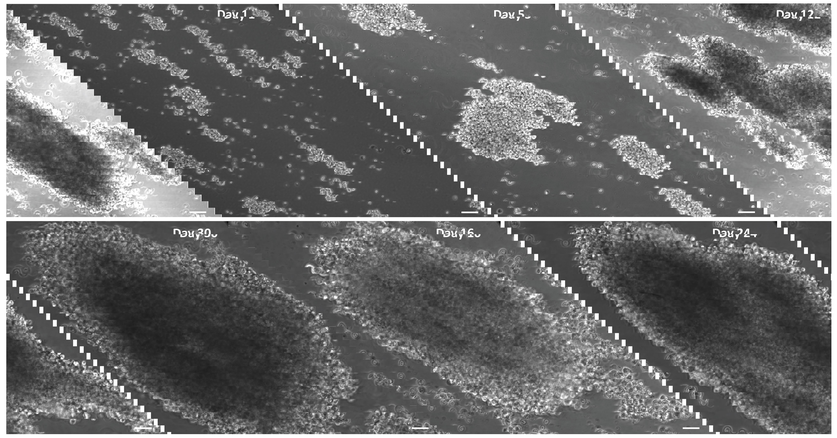


Figure 5: Long-term characterization of melanoma CTC cells. Cells were grown for 4 weeks resulting in large cell clusters. Cells were plated at $1x10^5$ cells/mL and imaged through growth period. Scale bars are 100 µm.

Table 1: STR baseline profile of MEL 167

STR Locus	Allele	STR Locus	Allele
D3S1358	18	D16S539	10,11
TH01	7	CSF1PO	10,11
D21S11	28,30	vWA	17,18
D18S51	13,14	D8S1179	13,14
D5S818	9,11	TPOX	8
D13S317	8,13	FGA	21
D7S820	8,10		

Conclusions

- MEL 167 (ATCC® CRL-3651™) cells, is the first widely commercially available circulating tumor cell (CTC) line, which is a promising tool for pre-clinical studies and monitoring metastatic disease.
- The common melanoma markers, MLANA and NG2, are present on the MEL 167 CTCs surface.
- The drug response to the BRAF inhibitor, Vemurafenib, of MEL 167 CTCs illustrates a relative similar drug profile as the melanoma model A375 (ATCC® CRL-1619™) and a higher drug sensitivity profile as the KRAS mutant-A375 isogenic line (ATCC® CRL-1619IG-1™).

References

Hong X., et al. The Lipogenic Regulator SREBP2 Induces Transferrin in Circulating Melanoma Cells and Suppresses Ferroptosis. Cancer Discov 11(3): 678-695, 2021. PubMed: 33203734

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