

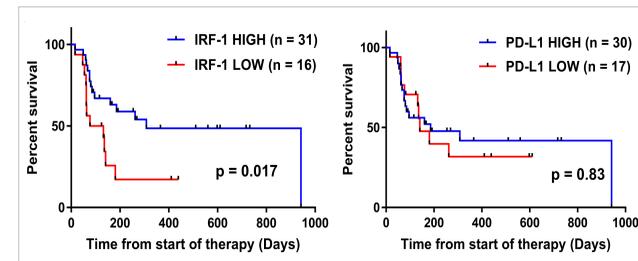
Simultaneous assessment of PD-L1 and IRF1 expression on breast cancer circulating tumor cells



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Background

There is a need for non-invasive predictive biomarkers of response to anti-PD1/PD-L1 therapies. Assessment of circulating tumor cells (CTCs) is a rational approach to non-invasive sampling of tumors to understand the potential response or non-response to anti-PD1/PD-L1 therapies. IFN-gamma signals through the JAK/STAT cascade to induce PD-L1 via the Interferon Regulatory Factor-1 (IRF1) transcription factor, and is a potent inducer of PD-L1 expression in tumor cells. Recent studies have shown that low or absent IRF1 expression can identify melanomas that lack IFN-gamma responsiveness, and that IRF1 can have a higher predictive value of response to anti-PD1/PD-L1 therapy than PD-L1 itself.



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Using CTC models and the RareCyte platform, we developed a multi-parameter assay that allows simultaneous PD-L1 and IRF1 assessment after CTC identification. We used this assay to better understand the relationship between IRF1 and PD-L1 expression in CTC model systems and applied this assay to blood samples from breast cancer patients.

Methods

Peripheral blood from normal donors or cancer patients under an IRB-approved protocol was collected into RareCyte blood collection tubes. PD-L1(+) and PD-L1(-) CTC models were created by culturing A549 overnight with or without 10ng/ml INF-gamma. The A549 cells were spiked into normal donor blood and buffy coats isolated from 7.5ml of blood by AccuCyte® separation and spread onto slides. MDA-MB-231 cells were used as a model to represent breast cancer cells that express PD-L1 in the absence of IFN-gamma signaling. Slides were stained with a 6-marker panel that included antibodies to pan-cytokeratin (CK), EpCAM, CD45, PDL1, IRF1, and a nuclear dye on the Ventana Discovery Ultra auto-stainer. Slides were scanned with CyteFinder® and CTCs identified by CK and/or EPCAM positivity and negative CD45 staining. Nuclear IRF1 and PDL1 expression was determined by mean fluorescence intensity in computationally estimated cell compartments. Currently, estimation of the nuclear compartment has a 5% failure rate, these data points were excluded from this analysis.

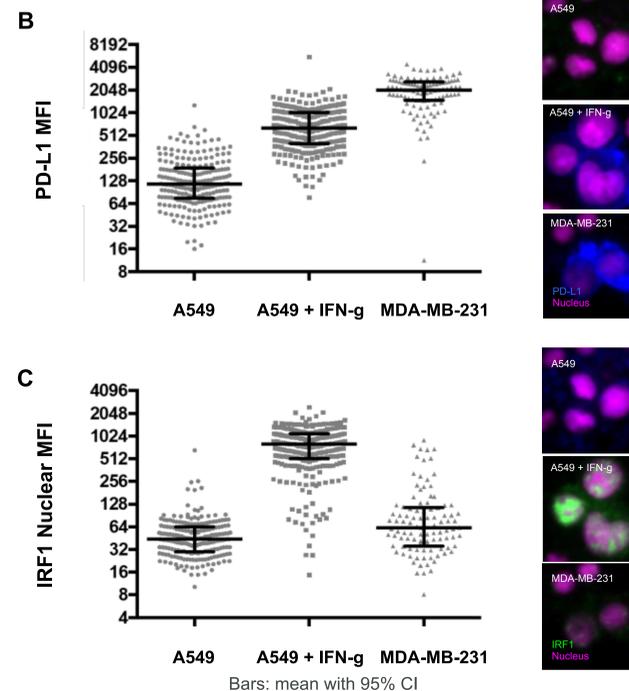
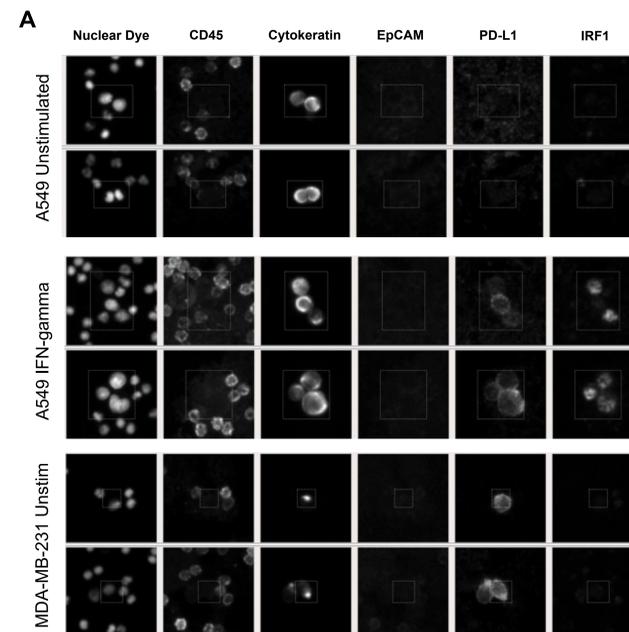


Figure 1. IFN-gamma induced expression of PD-L1 and nuclear IRF1 in spike-in CTC models. (A) 6-parameter analysis of cells detected using CyteFinder®. Mean fluorescence intensity (MFI) of PD-L1 (B) and nuclear IRF1 (C) on single cells that were identified.

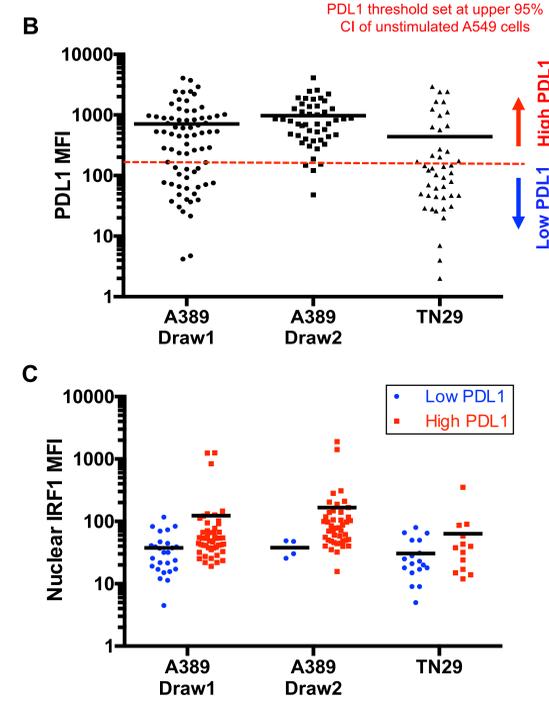
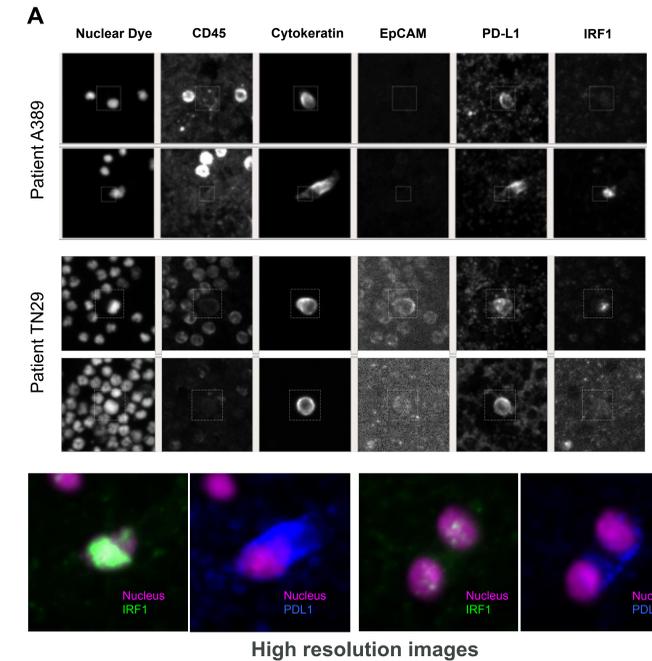
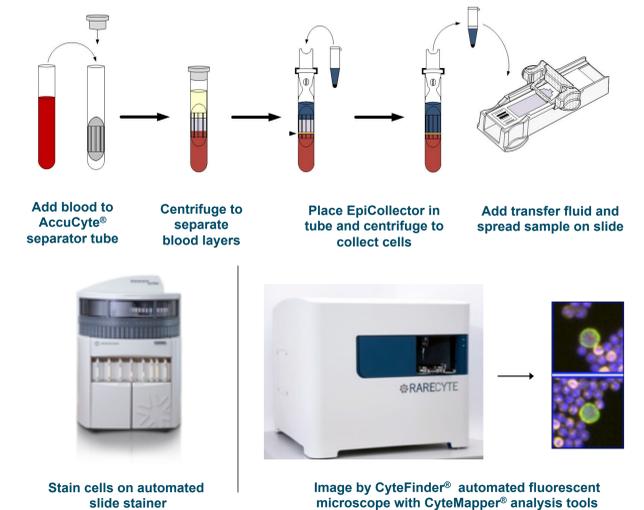


Figure 2. Expression of PD-L1 and nuclear IRF1 in breast cancer CTCs. (A) 6-parameter analysis of CTCs from two patients. (B) MFI of PD-L1 on the samples, with “high” threshold line set at upper 95% CI of unstimulated A549 cells. (C) Nuclear IRF1 MFI of single cells based on PD-L1 high/low stratification.

Sample preparation and analysis workflow



Results

Nuclear expression of IRF1 correlated with PD-L1 expression in IFN-gamma stimulated A549 cells. Unstimulated MDA-MB-231 cells expressed high levels of PD-L1, but this did not correlate with nuclear IRF1 expression. Breast cancer patient samples were identified having CTCs that expressed PD-L1. A PD-L1 mean fluorescence intensity (MFI) threshold was set at the upper 95% CI of unstimulated A549 cells to define “high” and “low” PD-L1 expression in the patient samples. The population of high-PD-L1 CTCs had higher nuclear IRF1 MFI than low-PD-L1 CTCs, with mean fold-increase of 2.7 (range from 1.4 to 3.3).

Conclusions

- In unstimulated A549s, PD-L1 staining and nuclear IRF1 staining were absent. In contrast, IFN-gamma stimulated A549s demonstrated PD-L1 staining and strong nuclear staining for IRF1.
- MDA-MB-231 had strong PD-L1 staining but low to absent nuclear IRF1 staining. This staining pattern may identify cancer cells with PD-L1 expression not due to IFN-gamma signaling.
- Identifying PD-L1 and IRF1 biomarkers on CTCs is feasible for non-invasive assessment. The cellular distribution of IRF1 together with PD-L1 expression may contribute additional predictive value to the assay.

Acknowledgements

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