Detection of Circulating Tumor Cells in Uveal Melanoma by the Photoacoustic Method

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ABSTRACT #5983

Circulating tumor cells (CTCs) have been shown to be a prognostic marker in breast cancer[1]. We hypothesize that circulating melanoma cell (CMC) detection could be utilized in the management of uveal melanoma, including early intervention. Prior methodologies for circulating uveal melanoma cell (CUMC) detection have been fraught with poor sensitivity, limiting their clinical utility[2]. Development of an improved method is necessary to establish the clinical utility of CUMC monitoring. Photoacoustics, also referred to as laser-induced ultrasound, is a novel platform for the detection and capture of CMCs. Photoacoustics uses short duration pulsed light to create ultrasonic acoustic waves in an optically absorbing medium, in this case melanin within melanoma[3]. As light is absorbed by irradiated chromophores, the optical energy gets converted into kinetic thermal energy trapped within the chromophore and subsequent thermal expansion ensues. Transient thermoelastic expansion of the absorbent cell results in the propagation of ultrasonic acoustic waves which can be detected and analyzed using a piezoelectric response mechanism. In addition, detected CMCs can be isolated by a two-phase flow cell separation technique[4]. Due to the low cost and melanoma specific capabilities of photoacoustics, we evaluated this technology for the purpose of CUMC detection.

Methods: Cells from uveal melanoma cell line UM002B, established at Thomas Jefferson, were titrated to various cell concentrations and analyzed in a neutral density solution utilizing the photoacoustic method. Uveal melanoma cells of differing concentrations were spiked into isolated healthy donor peripheral blood mononuclear cells (PBMCs) and healthy whole blood samples. PBMC isolates were analyzed for CUMCs.

Results: CUMCs were successfully quantified by the photoacoustic method including single cell detection. Recovery rates of cultured cells in a neutral density solution approached 25%. Recovery rates for CUMCs in whole blood averaged 10% of expected cell yield (56/540 cells detected) with a higher detection rate at lower cell concentrations. Photoacoustics offers a viable method for the detection of CUMCs with an accuracy that meets or exceeds previously reported CUMC yields. Studies analyzing CUMCs from patients with metastatic disease are ongoing.

METHODS

1. Uveal Melanoma Cell Line Sensitivity - Cells from a pigmented metastatic uveal melanoma cell line, UM002B, established at Thomas Jefferson, were titrated to various cell concentrations and analyzed in a control neutral density solution (82% Ficoll, 18% PBS) utilizing the photoacoustic method. 1cc cell samples were analyzed at a rate of 0.5µL/s. Cells were quantified in 2 minute intervals to show cell distribution (Fig. 4, 5). Titration testing was repeated 4 times (data not shown).

2. CUMC Measurements – Healthy donor peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples using standard Ficoll separation technique. 15,000 UM002B cells were spiked into isolated PBMCs and 15,000 cells were spiked into healthy whole blood samples prior to PBMC isolation. Isolates were suspended in 1cc of neutral density solution and CUMC detection was compared to healthy PBMCs (control) and to each other. Recovery rates were adjusted for PBMC signals (Fig. 6).

3. CUMC Recovery from Whole Blood – Varying concentrations of UM002B were spiked into healthy whole blood samples. PBMC components were isolated and suspended in 1cc neutral density solution. CUMC detection rates were adjusted for CUMC loss during PBMC isolation and for noise (Fig. 7). CUMC cells were isolated and stained with MART-1 antibody-FITC (Fig. 8).

PHOTOACOUSTICS

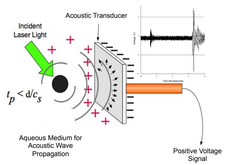

Fig. 1 – Schematic of photoacoustic method highlighting thermoelastic expansion of chromophore and signal transduction.

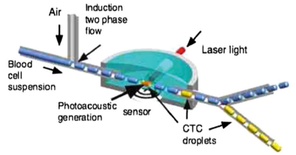

Fig. 2 – The photoacoustic flowmeter separates continuous flow of blood cells and immiscible air bubbles. Droplets containing pigmented cells are isolated for further analysis while the negative droplets are diverted for disposal.

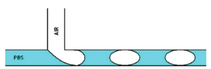

Fig. 3 – The introduction of two immiscible fluids, such as oil and water, produces two-phase flow under certain capillary conditions.

Photoacoustics uses short duration pulsed light to create ultrasonic acoustic waves in an optically absorbing cell, in this case melanin within melanoma[3]. As light is absorbed by irradiated chromophores, the optical energy gets converted into kinetic thermal energy trapped within the chromophore and subsequent thermal expansion ensues. Transient thermoelastic expansion of the absorbent cell results in the propagation of acoustic pressure waves defined by the condition

$$p = \tfrac{1}{2} (H_o)\, \mu_a\, \Gamma$$

where p is pressure, H_0 is radiant energy exposure (J/cm²), μ_a is the optical absorption coefficient of the cell, Γ is the Gruneisen coefficient, which denotes fraction of optical energy converted to acoustic energy. The resultant acoustic energy is transduced to a voltage signal indicating the presence or absence of an absorbent cell. CTCs can be quantified and subsequently isolated by a two-phase medium separation technique as illustrated above[4].

RESULTS

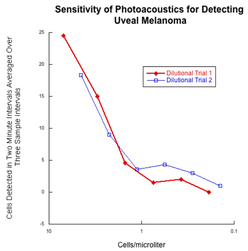

Fig. 4 – Linear regression plot of UM002B cell line serially diluted in 1cc neutral density control solution. Sensitivity below 1 cell/microliter on the order of **single cell detection**.

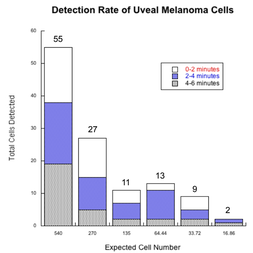

Fig. 5 – Uveal melanoma cells suspended in 1cc neutral density control solution. **Cell recovery ranges from 10-25%**. Detection limit 125 cell/mL.

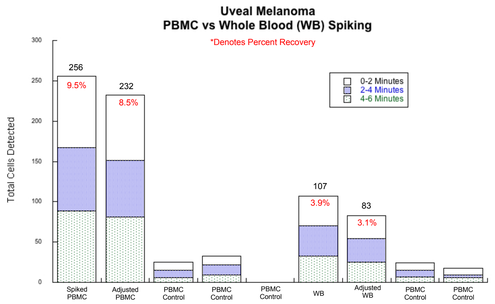

Fig. 6 – UM002B cell line spiked into isolated PBMCs and Whole Blood (WB) from single healthy donor. Samples suspended in 1cc neutral density solution and analyzed for CUMCs. Non-spiked PBMC controls shown. Spiked PBMC and spiked WB adjusted for baseline noise. Estimated **60% CUMC loss** during procedure to isolate PBMC from whole blood.

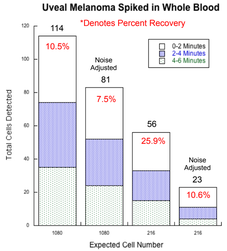

Fig. 7 – Various concentrations of UM002B cells spiked into healthy whole blood, isolated for PBMCs and analyzed. Expected cell number adjusted for 60% CUMC loss during isolation. **Ability to detect 1 CUMC in 10** present in noise adjusted whole blood sample.

Fig. 8 – Recovered CMCs stained with MART-1 antibody-FITC (green). CD45 antibody highlights surrounding leukocytes (red) under fluorescent microscope.

CONCLUSIONS

- Circulating Uveal Melanoma Cells (CUMCs) were successfully quantified by the photoacoustic method including single cell detection.
- Recovery rates of pigmented uveal melanoma cells suspended in a neutral density solution approached 25%.
- 60% CUMCs are lost during PBMC isolation. Superior isolation techniques should be investigated to increase CUMC recovery.
- Recovery rates for CUMCs in whole blood averaged 10% of expected cell yield (23/216 noise adjusted cell detection).
- The Photoacoustic Method offers a viable platform for the detection of CUMCs.
- Studies analyzing CUMCs from patients with metastatic disease are ongoing.

ACKNOWLEDGEMENTS

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