A Minimally Invasive Method (Liquid Biopsy) Enabling Detection of Rare Cells using Cell Enrichment and Immunohistochemistry

Continuous Innovation For Pathology

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Abstract

Introduction
Whole blood, serving as a "liquid biopsy", can provide a means of monitoring the status of a cellular or tissue-based event (e.g. cancer) using a minimally invasive needle stick of the patient.

Objective
To develop a protocol using a cancer cell enrichment method combined with an immunohistochemistry-based cell detection method.

Methods
Peripheral blood was spiked with cultured cancer cells. Cultured cancer cells were enriched by depleting white blood cells (WBC) using antibody coated magnetic beads and red blood cells (RBC) were depleted using a chip-based cell enrichment method. Cells were stained including Benzidine Wrights Giemsa (BWG) and immunohistochemistry (IHC) protocols.

Results
Using 1 mL of whole blood, the chip-based protocol enriched the target cells 100-fold and achieved greater than 40% recovery rate. The target cells were identified using cell-specific biomarkers on an automated IHC staining system.

Conclusion
Using a novel target cell enrichment protocol in combination with IHC, liquid biopsies may provide an alternative method to detect rare cells, which may support cancer identification and monitoring.

Materials and Methods

Cancer Cell Lines
Human cancer cell lines (breast, BT-474; and colorectal, DLD-1) were cultured under the conditions recommended by the supplier (ATCC, Manassas, VA).

Automated Cancer Cell Enrichment
Using spiked cultured cancer cells as a model, cancer cells were fluorescently tagged using the vitality stain acetylmethoxyester-calcein, (Calcein-AM, eBiosciences, San Diego, CA). The unfixed, fluorescently labeled cancer cells were spiked into peripheral whole blood.

A cell enrichment kit was used to enrich the cancer cells (RedSift Cell Processor System, AVIVA Bioscience, San Diego, CA). Antibody-coated magnetic beads were incubated with the spiked blood. White blood cells were magnetically negative-depleted. The supernatant with unbound cells was recovered and then filtered to deplete red blood cells.

Identification of Enriched Cancer Cells Using Flow Cytometry
The recovered cells were analyzed using a Cytomics FC500 flow cytometer (Beckman Coulter, Indianapolis, IN) to detect cancer cells (Calcein-AM) or white blood cells (CD45-APC, Becton Dickinson, San Diego, CA).

Benzidine Wrights Giemsa (BWG) Staining of Peripheral Blood
The recovered blood cells were:

- Cytocentrifuged onto slides using Sakura® Cyto-Tek® 2500 Cytocentrifuge (Sakura Finetek USA, Torrance, CA)
- Fixed with methanol for 5 minutes
- Incubated with 1% benzidine for 1.5 minutes
- Incubated with peroxide solution for 1.5 minutes
- Rinsed
- Incubated in Wrights Giemsa solution for 10 minutes
- Rinsed and air-dried

Identification of Cancer Cells Using IHC
An antibody cocktail was used to detect cancer cells by IHC using the Tissue-Tek Genie® Advanced Staining System (under development, Sakura Finetek USA, Torrance, CA).

The slides were coverslipped using Tissue-Tek Film® Coverslipper (Sakura Finetek USA, Torrance, CA) and images were taken using VisionTek® M6 Digital Microscope at 40x (0.138 µm/pixel, Sakura Finetek USA, Torrance, CA).

Results

Figure 1. Example of white and red blood cell depletion (10^{1-2} for WBC's and 10^{3-4} for RBC's) using magnetic beads and filtration. Whole blood results before (left) or after (right):

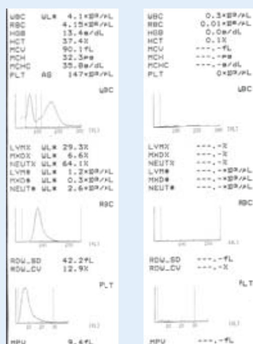

Figure 2. Example of cultured cancer cell recovery after WBC and RBC depletion. Spike control sample (left) compared to cancer cells recovered (right):

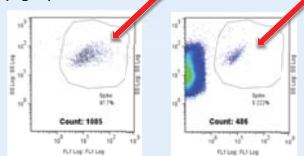

Table 1. High target cell recovery was observed using automated cell enrichment:

Cell type	Removal	Recovery
White blood cells	~10^{1-2}	Not applicable
Red blood cells	~10^{2-3}	Not applicable
Cultured cancer cells	Not applicable	>40%

Figure 3. Instruments used in this study: RedSift Cell Processor (left) and Tissue-Tek Genie Advanced Staining System (right):

Figure 4. Breast cancer cells (BT474) were recovered and BWG stained:

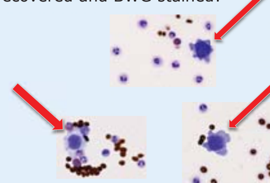

Figure 5. Breast cancer cells (BT474) were recovered and stained using IHC:

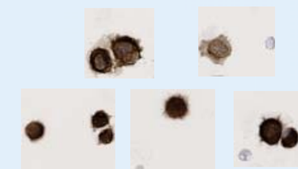

Figure 6. Detection of cultured breast cancer cells using short antigen retrieval (AR) times:

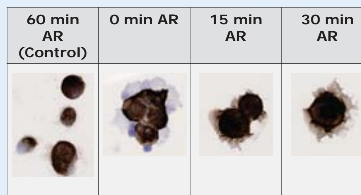

Figure 7. Cultured colorectal cancer cells (DLD-1) were recovered and BWG stained:

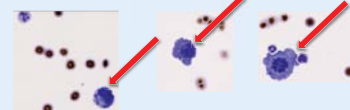

Figure 8. Cultured colorectal cancer cells (DLD-1) were recovered and stained using an IHC antibody cocktail:

Conclusions

- It was possible to enrich spiked cancer cells from peripheral whole blood
- Antibody cocktail to epithelial antigens was able to detect spiked cultured cancer cells
- An automated cell enrichment and identification procedure reduces the potential for human error for these complex procedures
- "Liquid biopsy" may be a valuable tool to identify rare circulating cancer cells
- "Liquid biopsy" processed using an automated enrichment and identification method contributes to high quality results

Contact Information

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