Liquid Biopsy: A Minimally Invasive Method Enabling Prenatal or Cancer Diagnostics and Monitoring of Residual Disease using IHC

Douglas T. Yamanishi PhD[1], Paul G. Hujsak[2], Sara F.S. Hansen[2], António Guia PhD[2], Ky Truong[2], Mark Sarinana[2], Cliff Hom[1], Adam Hammer[1], Claudio Scancich[1] and Erico von Bueren PhD MD MOR[1]

[1]Sakura Finetek USA, Inc., Torrance, CA; [2]Aviva Biosciences, Inc., San Diego, CA

Continuous Innovation For Pathology

Introduction

A minimally invasive method of monitoring disease status before, during and after treatment benefits both patient and physician. Whole blood serving as a "liquid biopsy" can provide a means of monitoring the status of an event (e.g. pregnancy or cancer) using a minimally invasive needle stick.

A simple blood draw is the catalyst for retrieving rare, clinically relevant cells such as tumor cells (cancer) or fetal cells (pregnancy), which have been shed and circulate freely within the bloodstream. These circulating cells have implication in diagnosis and minimal residual disease (MRD) monitoring. The enrichment and detection of rare circulating cells may provide a valuable, more tolerable alternative to an invasive solid tissue biopsy procedure, like amniocentesis or chorionic villus sampling (CVS).

Materials and Methods

Manual Fetal Cell Enrichment and Identification

A manual procedure (using magnetic beads and filtration chips) was developed after evaluating different enrichment technologies (Table 1) to enriched fetal cells from whole peripheral blood (Advanced Biosciences Resources, Alameda, CA with IRB approved protocol). Fetal nucleated red blood cells (nRBCs) were detected using manual immunohistochemistry (IHC) in combination with fluorescent *in situ* hybridization (FISH) using chromosome enumeration probes, CEP X Aqua and CEP Y Orange (Abbott Molecular, Des Plaines, IL; Schueler, P.A., et. al. Placenta 22: 688, 2001). Slides were coverslipped and images were taken using a fluorescent digital microscope.

Figure 1. Manual enrichment and identification of fetal cells. Male or female fetal cells (cells with labeled green cytoplasm and blue nuclei) are identified from maternal cells (cells with blue nuclei)

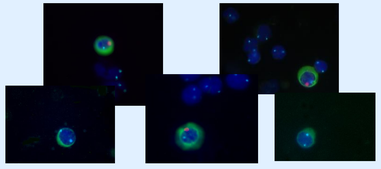


Table 1. Target cell recovery using different cell enrichment technologies.

Technology	Target cell recovery	Comments
Density gradients	< 80%	Target cells can be present in multiple density layers.
Centrifugation	80-90%	With peripheral blood, braking or slight shaking of the tube can result in cell loss.
Magnetic bead capture	70-90%	Recovery depends on antibody and magnetic bead capture.
Dielectrophoresis biochip	< 80%	DEP with sugar-based suspension medium. Observed cell lysis and target cell loss.
Filtration chip	> 90%	Recovery is dependent on slit or hole width. Optimization required for target cell type.

Cancer Cell Lines

Human cancer cell lines (breast, BT-474; colorectal, DLD-1; lung, H526) were cultured under the conditions recommended by the supplier (ATCC, Manassas, VA).

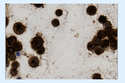
Automated Cancer Cell Enrichment and Identification

Using the fetal cell enrichment procedure as a model for cancer cell enrichment, cultured cancer cells were spiked into whole peripheral blood. An automated cell enrichment kit was used to enrich the spiked cancer cells (AVIVA Bioscience, San Diego, CA). The recovered cells were put onto slides and fixed. A new antibody cocktail was used to detect cancer cells by IHC and counterstained with hematoxylin to identify non-target cells. The slides were coverslipped using Tissue-Tek Film® Coverslipper (Sakura Finetek, Torrance, CA) and images were taken using VisionTek® Digital Microscope (Sakura Finetek, Torrance, CA).

Results

Figure 2. Breast (BT-474) or lung (H526) cancer cells can be detected with antibody cocktail.

Breast cancer



Lung cancer

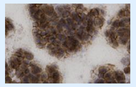
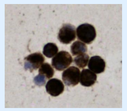


Figure 3. Colorectal (DLD-1) cancer cells can be detected with two antibodies in antibody cocktail.



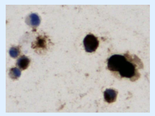
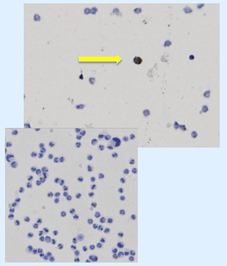


Figure 4. Automated enrichment and identification of colorectal (DLD-1) cancer cells spiked into blood.

Spike



Enriched

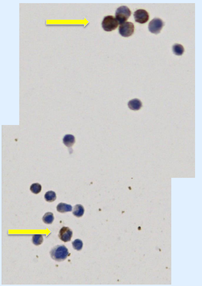


Table 2. High target cell recovery was observed using automated cell enrichment.

Cell type	Removal	Recovery
White blood cells	~10^2-10^4	~80-90%
Red blood cells	~10^2-10^4	>90%
Cultured cancer cells	Not Applicable	>70%

Conclusions

- It was possible to enrich rare target cells from peripheral blood (e.g. fetal cells or cancer cells).
- Antibody cocktail to fetal or epithelial antigens was able to detect circulating fetal nRBCs or spiked cultured cancer cells.
- The automated cell enrichment and identification procedure had similar target cell recoveries compared to manual procedure, thereby reducing the potential for human error with complex procedures.
- "Liquid biopsy" may be a valuable alternative to invasive procedures to identifying rare circulating target cells.
- In the future, "liquid biopsy" and automated enrichment and identification instruments may help a clinician monitor patient status.

Contact Information

Booth #101

Douglas Yamanishi, Ph.D.
Senior Marketing Manager, Advanced Staining
Sakura Finetek USA, Inc.
1750 West 214th Street
Torrance, CA 90501
310 972-7800 x8091
DYamanishi@SakuraUS.com

0007702-01 Rev.A