A Novel Method for Placental Evaluation: Isolation of Intact Circulating Trophoblast Cells from Maternal Circulation

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Abstract #360

PURPOSE

We aim to test the hypothesis that gene expression profiles of placental cells in maternal circulation can be used as a biomarker to predict risk of stillbirth and other adverse pregnancy outcomes. As a foundational step in this effort, we developed a method to isolate individual, intact circulating trophoblasts (CTBs) from maternal circulation.

BACKGROUND

Trophoblasts first enter the maternal circulation early in gestation during the process of placental implantation, and transfer continues throughout pregnancy. CTB counts can differ between normal pregnancy and pregnancies with complications associated with placental dysfunction, e.g. preeclampsia (PE) and fetal growth restriction (FGR).[1,2] Isolated CTBs are effectively liquid biopsy samples with transcripts and proteins of placental origin. Understanding normal transcriptional profile of CTBs allows investigation of changes to that profile in disease, with significant potential for improving understanding and prediction of adverse pregnancy outcomes.

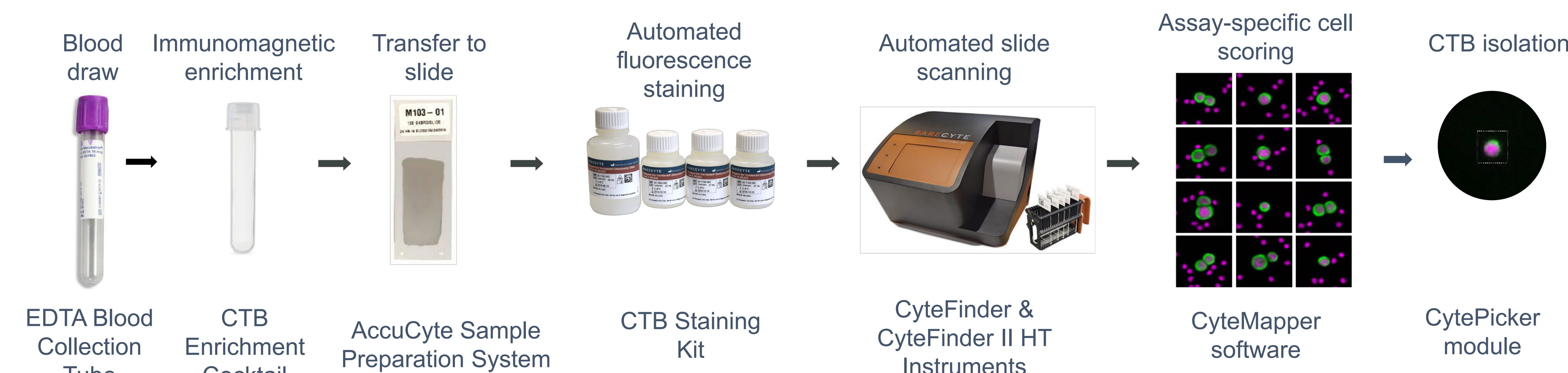
METHODS

After informed consent, peripheral blood samples were collected from a cohort of women in the third trimester of singleton pregnancies. Using AccuCyte®, an unbiased density-based method for collecting nucleated cells from whole blood, we collected nucleated cells and performed immunomagnetic bead enrichment using a cocktail of markers, followed by transfer to slides. We then stained for Hoechst (nucleus), pan-cytokeratin (CK, trophoblast marker), TMX (supportive trophoblast marker), and CD45 (WBC, exclusion marker). Slides were imaged with CyteFinder®, an automated, multiparameter immunofluorescent (IF) microscopy system that applies machine learning algorithms for cell identification. Each slide contains millions of cells, so we utilized novel scanning and analysis algorithms to identify candidate CTBs. CTBs were then individually isolated using the CytePicker® Retrieval Module.

RESULTS

An overview of the clinical characteristics of our pregnancy cohort is shown in **Table 1**. Most pregnancies were uncomplicated at the time of the blood draw and remained uncomplicated throughout pregnancy. In one case, a participant development subsequent PE, and in another, the participant had FGR at the time of blood collection. Candidate CTBs, Hybrid cells (HYB), and Indeterminate cells (IND) were defined as CD45-/CK+, CD45+/CK+, CD45-/CK+/uncharacteristic cellular features (nuclear morphology, staining pattern, etc.), respectively, and counts of each cell type per ~30 mL blood are shown in **Table 1.**

SAMPLE ANALYSIS WORKFLOW



CIRCULATING TROPHOBLASTS AND RELATED CELLS

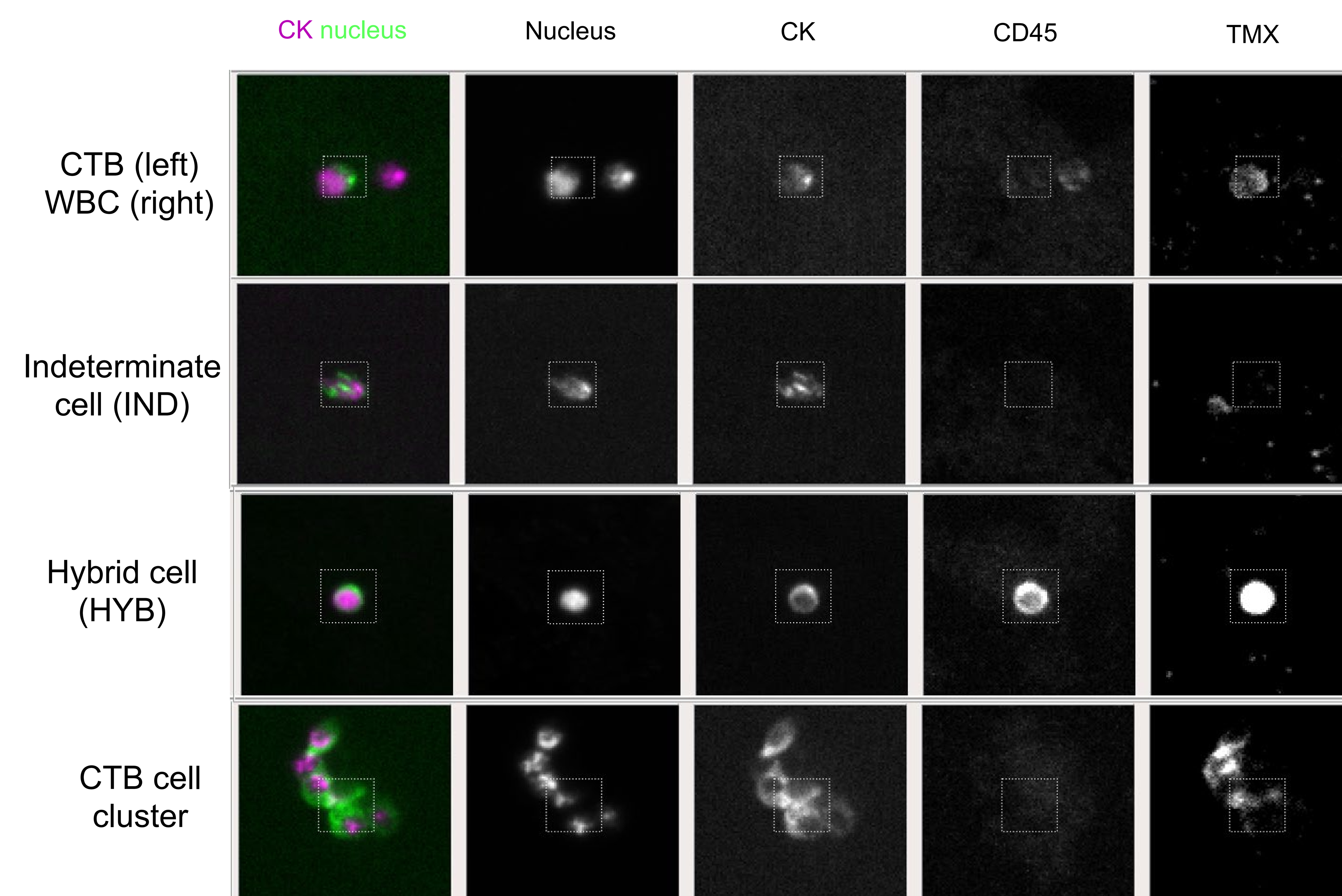


Figure 2. Representative images across all fluorescence channels showing circulating trophoblast, white blood cell, indeterminate cell, hybrid cell and CTB cluster.

Figure 1. RareCyte platform workflow. Blood is collected into EDTA Blood Collection Tubes (BCTs). After collection of nucleated blood cells and immunomagnetic bead enrichment, cells are processed to slides using the AccuCyte Sample Preparation System. Slides are stained with assay-specific RareCyte staining kits for the markers indicated. Slides are scanned using the CyteFinder Instrument and images are analyzed using CyteMapper® software and analysis tools. All cells are analyzed by a trained reviewer. Selected cells are retrieved from the slide with the CytePicker automated module within the CyteFinder instrument.

Table 1. Summary data by study participant. Gestational age and clinical characteristics for pregnancy cohort participants are shown

Patient ID	GA at Draw	# CTBs	# IND Cells	# HYB Cells	Clinical Status
P-056	25w5d	0	1	3	Normal
P-052	26w0d	0	3	0	Normal
P-047	26w5d	4	7	3	Normal
P-043	27w0d	3	3	3	Normal
P-042	28w0d	3	2	0	Normal
P-054	28w0d	7	1	0	Normal
P-041	28w1d	4	1	NA	Normal
P-044	28w2d	0	0	0	Normal
P-049	30w0d	1	0	2	Normal
P-050	30w3d	2	1	3	Normal
P-046	31w1d	6	0	0	Normal
P-045	31w4d	6	0	3	Normal
P-051	31w6d	2	3	4	Normal
P-053	32w3d	3	8	0	Normal
P-003	36w1d	1	7	NA	Normal
P-040	36w1d	5	0	0	Normal
P-057	36w1d	0	15	0	Normal
P-001	36w3d	3	1	NA	Normal
P-035	37w3d	6	2	NA	Normal
P-019	25w5d	9	11	NA	Subsequent PE
P-048	30w1d	2	1	0	FGR
P-060	35w1d	17	19	0	PE

DISCUSSION

We have established a novel and reproducible method for identification and isolation of intact CTBs from maternal circulation in the third trimester. Using this approach, we can now conduct robust studies of CTB enumeration, standardized cytological evaluation, and, ultimately, transcriptional profiling across GA in uncomplicated pregnancies and in the setting of adverse pregnancy outcomes. Ultimately, we aim to use this technology, including evaluation of the transcriptome of CTBs via RNA-sequencing, to identify and enable intervention in women at high risk of placental dysfunction and related disorders including stillbirth.

ACKNOWLEDGEMENT

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