1. INTRODUCTION

- Circulating tumor cells (CTCs) detach from solid tumors and enter the blood stream. CTCs play a crucial role in cancer metastasis.
- Vortex technology is a label-free microfluidic platform for the enrichment of CTCs with high recovery, high purity, and simple processing. Successful CTC isolation has been demonstrated in various human cancers, including breast cancer.
- There is a growing interest in the use of murine models to study CTCs, but this application faces multiple challenges:
  - Few CTCs are present among millions of white blood cells (WBCs) and billions of red blood cells (RBCs).
  - Blood volumes are small (200-800 µL).
- Previous studies showed that CTCs could be isolated from tumor xenografts using Vortex technology.
- Here, we present workflow optimization of mouse blood processing using the VTX-1 automated instrument and some applications.

2. MATERIALS AND METHODS

3. WORKFLOW OPTIMIZATION FOR VTX-1 PROCESSING

3.1 Blood dilution optimization

- 200 cells spiked in 500 µL blood and processed with manual setup using a range of blood dilutions (10X, 20X, 40X).
- Successful isolation of cancer cells spiked in mice blood with Vortex technology.
- Lower cell recovery with a 10X blood dilution.

3.2 Sample recycling by the manual setup

- 500 cells spiked in 500 µL blood and processed with manual setup.
- Efflux was reprocessed through the Vortex chip (1 processing event = 1 cycle).
- Recycling the efflux increases cell recovery: 93% of total cells captured are collected in the first 2 cycles. A 2-cycle protocol will be selected for the next steps.

3.3 Testing using the VTX-1 instrument

- 200 cells spiked in 600 µL blood and processed with VTX-1, 2 cycles.
- Improved recovery (61.30%) of cancer cells spiked in mouse blood with VTX-1 instrument.

4. APPLICATIONS WITH VARIOUS ASSAYS

4.1 Clonogenic assay

- Protocol

4.2 3D spheroid cell invasion assay

- Protocol

4.3 Patient-Derived Orthotropic Xenograft (PDX) CTCs captured by VTX-1

- Live minced pieces of tumor generated from a Stage II patient with triple negative (ER-/PR-/HER2-) breast cancer were implanted into the mammary fat pads of two immunodeficient (NOD scid gamma) mice.
- PDX tumors were grown and blood (750 µL) was collected via cardiac puncture from each PDX model and processed through the VTX-1 instrument (2-cycle protocol).
- Isolated cells were stained with anti-EpCAM (clone 3B11) + Vimentin (clone Vim9) antibodies + DAPI.

5. CONCLUSIONS & FUTURE DIRECTIONS

- The workflow was optimized to transition from an R&D manual setup to an easy, automated platform for isolating cancer cells from murine blood samples.
- After processing through Vortex technology, the cells maintain their clonogenicity as well as their ability to form 3D spheroid structures and to invade extracellular matrix.
- CTCs were successfully isolated from blood from two PDX samples with good purity (> 200 contaminating WBCs/sample).
- A total of 50 PDX samples have been processed through Vortex technology and CTCs at different stages of EMT, including CTC clusters, isolated.
- Vortex technology can be used as a platform to study CTCs in murine models of breast cancer, and can be potentially adapted to other types of cancer xenografts (e.g., prostate, lung, colorectal).

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