Conference Abstracts

Rapid, Reproducible, Rare Cell 3D Expansion (R3CE®) of CTC spheroids from peripheral whole blood from patients with metastatic solid tumors

Yung-Hao Lin, PhD[1], Aurin Liu, BS[1], Ange Bair, PhD[1], Jia-Yang Chen, PhD[1], Ying-Chih Chang, PhD[1], Mark D. Pegram, MD[1a]

[1] Binaytara Foundation, 3380 146th Pl SE Suite 330, Bellevue, WA

Corresponding author

[a] Mark D. Pegram, MD, Binaytara Foundation, 3380 146th Pl SE Suite 330, Bellevue, WA, Email: mpegram@stanford.edu

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Background

We previously measured CTCs in transgenic *Neu*N (NTTg) mice that spontaneously develop breast cancer [Tsai W-S, et al. Cancers 13:3294(2021)]. In this model, CTC counts rose 1±1.6 to 16±9.5 per 75μL, increasing with tumor progression. CTC tumorigenesis was confirmed by re-implantation into non-tumor-bearing mice. Doppler ultrasonography showed stronger correlation between CTCs and tumor vascular density ($p< 0.01$), than with tumor volume (p=NS). We next purified CTCs from primary and metastatic tumors in mouse models to demonstrate phenotypic and multi-omic analyses of CTCs. CTCs and metastatic cells, but not primary tumor cells, shared stochastic mutations and similar hypomethylation levels at transcription start sites. CTCs and metastatic tumor cells shared an epithelial-mesenchymal transcriptome (EMT) state with reduced adhesive and enhanced mobilization characteristics. These multi-omic results reveal that CTCs resemble metastatic tumors, establishing CTCs as a potential *ex vivo* tool for precision oncology [Chen J-Y, et al. iScience 25:105081(2022); Yeh PY, et al. J Mater Chem B 11(34):8159-69(2023); Tsai HA, et al. Biomacromolecules 13(11): 3483-93(2012)]. Thus far, CTC platform technologies have failed to deliver critical information to medical oncologists [Morgan P, et al. Nat Rev Drug Discov 17(3):167-81(2018)]. CTC measurements have been limited by low sensitivity, a paucity of cells recovered (CellSearch® cutoff is 5 CTCs/7.5mL whole blood – too few to measure clinical phenotypes of interest by molecular methods), and failure to capture cells that have undergone EMT.

Methods/Results

Here we present a novel CTC platform technology: 3D Rapid, Reproducible, Rare Cell 3D Expansion (R3CE), capable of high yield (sufficient for NGS, as well as IHC, FISH, RNAseq, etc.) isolation and *ex vivo* expansion of CTCs (grown as tumor spheroids) from <10mL of peripheral blood, suitable for further multi-omic diagnostic characterization of human solid tumors. We hypothesize R3CE CTC technology can replace tissue biopsy as a DNA source for NGS in metastatic solid tumors.