Utilizing Vortex Chip for Enumeration and Determination of Single-Cell Heterogeneity of Circulating Tumor Cells in Prostate Cancer

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Background: Circulating tumor cells (CTCs) are cells that break away from either the primary tumor or metastatic sites and circulate in the peripheral blood. We developed a CTC capture technology known as Vortex Chip, which allows for rapid isolation of highly purified CTCs in a matter of minutes. Moreover, because the Vortex Chip isolates CTCs based on their size, our chip does not require antibody based capture that can potentially bias the isolation of CTC subpopulations to cells that express a specific surface marker (i.e. epithelial cell adhesion molecule [EpCAM]). We had previously designed the Vortex HT chip for higher sample throughput and have utilized this chip for capture of patient samples. We have also designed a new version of the Vortex Chip, Vortex Chip HE (i.e. **H**igher **E**fficiency), that manifests increased capture efficiency, which has improved our ability to successfully and more consistently perform molecular studies such as exome and gene specific sequencing. We also assessed the potential for epithelial-mesenchymal transitions in the tumor through immunostaining of the isolated CTCs.

Methods: The Vortex Chip was fabricated using standard lithographic techniques with polydimethylsiloxane (PDMS) polymer. Samples were run using a syringe pump setup to deliver blood to the chip at varying flow rates, with large cells (including CTCs) trapped in vortices in each chamber. Cells trapped in vortices were released using PBS rinse buffer. These cells are subsequently subjected to immunofluorescence and downstream nucleic acid analyses. For example, we performed total RNA amplification and RNA sequencing utilizing the Quartz-Seq methodology with reads performed on an Illumina HiSeq instrument, and have also performed whole genome amplification and whole exome sequencing of pooled prostate CTCs.

Results: With the HT chip design, we achieved an overall capture efficiency of 15-20% per run based on control cancer cell line experiment, with enrichment of CTCs by a 10,000-fold magnitude. With the HE chip design, we could increase capture efficiency to up to 30% per run. Factors affecting the capture efficiency included the size and shape of the channels. We were also able to improve overall capture efficiency to over 50% by running samples multiple times through the chip. Previously we had utilized the Quartz-Seq protocol for transcript amplification and subsequent RNA sequencing and we found that RNA quality is variable from CTCs. We have now switched to exome sequencing as the DNA is of higher quality in the CTCs. We are working to develop protocols for high quality whole genome amplification with reduced coverage gaps. Once fully optimized, we will compare sequencing results from CTCs to that of matched tumor specimens obtained from metastatic foci and analyzed in the context of the SU2C study.

Conclusions: The Vortex Chip has excellent capture efficiency with high purity, and allows us to capture CTCs rapidly, within thirty minutes. We have established poof-of-principle evidence that next generation sequencing can be performed on captured CTCs. Further optimization of our chip and techniques to obtain exome sequences in addition to enhancing analysis algorithms will improve read coverage. Ultimately, our CTC technology and strategies will provide additional insight into heterogeneity in prostate cancer as well as offer the promise of precision/individualized treatment.

Conflicts of Interest: None declared

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