Improved Methods for Capturing and Analyzing Circulating Tumor Cells

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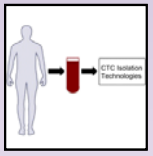
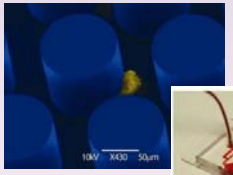
Circulating Tumor Cells (CTCs) as a Cancer Diagnostic

- Important to isolate and enrich
  - Facilitates early detection in malignant disease patients
  - Enables more effective treatment
- Limitations of CTC isolation
  - Requires large processing volumes (7 ml of blood)
  - Requires long processing times
  - Causes phenotypic shift or even loss of viability
- Emerging methods employ microfluidic technologies
- Once captured, CTCs are analyzed to tailor treatment
- Project aims to develop a rapid transgene assay to evaluate enriched CTCs

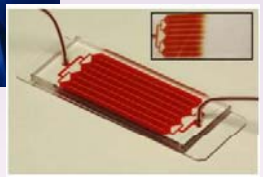


Obtaining CTCs from patients

Veridex CellSearch system used to isolate CTCs



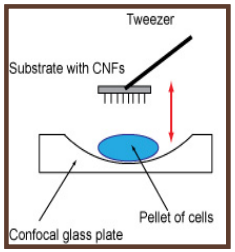
From Stott et al (2010), PNAS



Microfluidic platforms used to capture CTCs

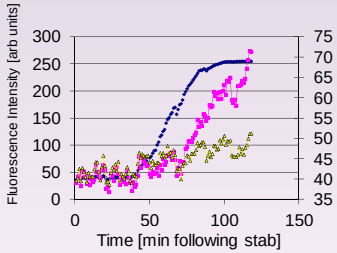
Rapid Analysis of Enriched CTCs

- Breast cancer cell line (called 'MCF-7') used as CTC surrogate
- Cell culture
  - Media: Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin, non-essential amino acids, L-glutamine, and sodium pyruvate
  - Cells fed and harvested 2-3 times a week
- Analysis of CTCs
  - Harvested via trypsinization or chelation techniques
  - Pelleted by centrifugation (60xg, 10 min)
  - Nanofiber chip method (impalement) exploited for rapid delivery of transgene DNA
  - Yellow fluorescent protein (YFP) plasmid grown in e. coli and isolated using Qiagen MiniPrep
  - Microscopic tracking establishes transgene expression timeframe



Impalement process

Impalement gene delivery method



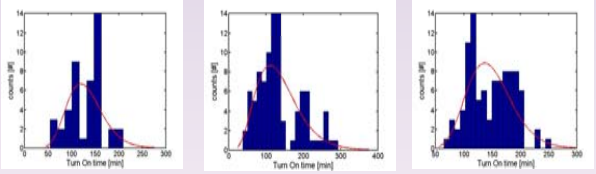
Raw data of YFP fluorescence vs. time

Results and Conclusions

- MCF-7 cells maintain viability following rapid transgene technique
- YFP rapidly expressed and observed using inverted fluorescence microscopy and automated image capture (1 min intervals)
- Earliest transgene expression observed within 50 minutes
- Average transgene expression observed ~130 minutes
- Timeframes are consistent with nuclear delivery

Next Steps

- Research telomere binding protein (TRF2) mislocalization in metastatic breast cancer cells
- Conducting delivery and expression of TRF2-GFP provides visualization of TRF2 localization within cells
- Rapid transgene studies assess cells metastatic potential
- Provides means of assaying cellular response to chemotherapy



Histograms of MCF-7 cells fluorescent turn on time (in minutes) for rapid transgene expression of YFP plasmid

1. Minetta C. Liu, P. G. (2009). Circulating Tumor Cells: A Useful Predictor of Treatment. Journal of Clinical Oncology, 5153-5158.
2. Shannon L. Stotta, b. C.-H. (2010). Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. PNAS, 18392-18397.
3. Sunitha Nagrath1*, L. V. (2007). Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature*, 1235-1238.

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