Development of optimized 3D migration and invasion assays for siRNA screening

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Abstract

Many high-throughput screening approaches have been developed to determine small molecules that inhibit the migration of cells across 2D surfaces. However, often agents discerned by this approach have minimal effect on cell invasion in the more complex extracellular matrix found *in vivo*. We sought to perform an siRNA screen in 3D collagen matrices that had more relevance to *in vivo* migration using breast cancer cells. This will further contribute to understanding the genes involved in breast cancer metastasis and the signaling pathways cancer cells employ to interact with the extracellular matrix. In order to test which genes are involved in invasion we have used a variety of invasion assays that were compared side by side. We tested length of assay, serum-conditions, cell-seeding numbers, ease of assay, and end result monitoring. After contrasting various classic and commercial assays, we determined that iuvo microchannel 192-well format from BellBrook Labs (Madison, WI) works the best to monitor genes whose knock-down perturbs 3D cell migration. Results of the screen are in process.

Introduction

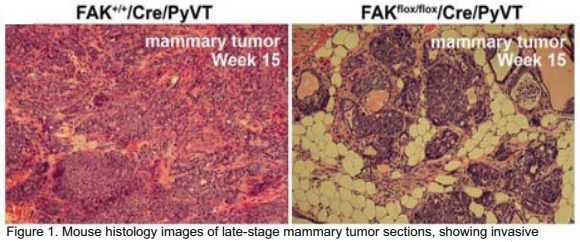


Figure 1. Mouse histology images of late-stage mammary tumor sections, showing invasive phenotype of FAK-positive cells and the non-invasive phenotype of FAK-negative cells[1]

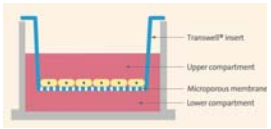
Classical studies on molecular mechanisms have involved investigating cancer cells in 2D tissue culture environments. However, it is becoming clear that this approach loses many aspects of the *in vivo* environment that cells encounter, including the 3D geometry of the microenvironment. In order to understand how cells interact with their environment, migration will be assessed in the context of 3D collagen matrices. Dense breast tissue is associated with increased stromal collagen, which has been correlated with an increased risk for breast cancer[2]. Moreover, when breast carcinoma cells invade and metastasize, they do so through the collagen rich stroma. For these reasons, I will screen which genes influence migration in cancer cells upon encountering of a three-dimensional collagen matrix. Genes that we have chosen to be part of this screen resulted from a previous microarray that feature the knockout of focal adhesion kinase (FAK) in a mouse breast cancer model. FAK -/- tumors proliferated at the same rate as wild type but failed to metastasize suggesting that proteins regulated by FAK are also critical for invasion[1]. This set of genes was contrasted with a published dataset with genes involved in metastasis in PyVT mice-derived tumors[3]. In order to test their role in migration I have been working with a high-throughput invasion assay to help discern which genes, by knockdown, are vital for invasion using a highly invasive variant cell line (MCF10Ca-1d).

Tested Assays

ORIS™ Assay by Platypus Technologies

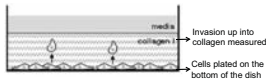
Extracted from www.platypustech.com

Transwell® from Corning



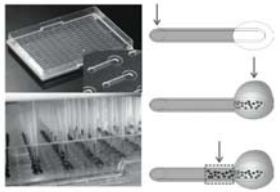
Extracted from www.corning.com

"Reverse" invasion assay



Adapted from Dr. John Condeelis[4]

iuvo microchannel by BellBrook Labs



Extracted from www.bellbrooklabs.com

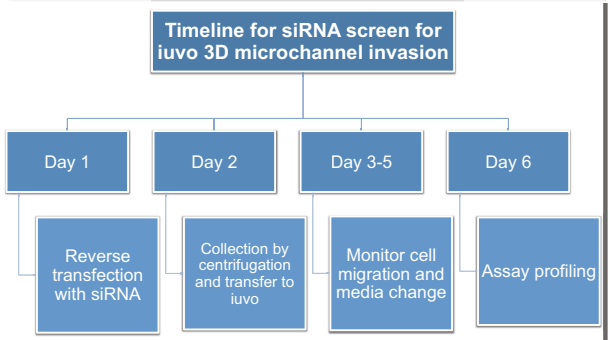
Invasion Assay Summary of Pros

- ✓iuvo, Reverse invasion and ORIS assays provide live monitoring of cell migration
- ✓iuvo offers less handling of sample (siRNA and cell seeding) due to robot automated system by CyBi®-Well
- ✓iuvo bypasses variability and human error
- ✓iuvo, Reverse invasion and ORIS assays could be analyzed for cell death vs. non-migratory profiling
- ✓iuvo allows less amount of siRNA used in one run and allows samples in quadruplicate for statistical significance analysis

Invasion Assay Summary of Cons

- •Transwell allows only end point invasive cell number. Also, does not offer the quantification between dead cells and non-migratory
- •iuvo does not offer the capability to experiment with different collagen gel concentrations
- •Transwell and Reverse invasion assays require higher cell number and thus more siRNA needs to be dispensed

High-throughput screen timeline



Assay profiling

1. Distance measurement of cell migration in 3D
2. Quantitative cell number and distance travel data in different planes
3. Dead vs. non-migratory using dyes at end point
4. Live monitoring of assay by light microscope

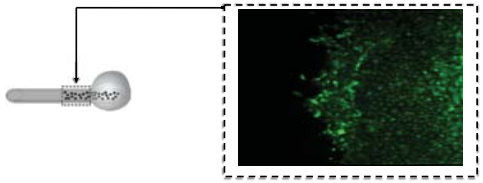


Figure 2. Representative image of MCF10Ca-1d live-cell stained with Calcein AM in iuvo microchannel after 5 days. 2000 cells were initially seeded in 5µL of media with 2µL of 10% serum media in the input port.

References

1. Provenzano, PP. *et al. AJP* **173**: 1551-65 (2008)
2. Boyd, NF, *et al. Cancer Epidemiol Biomarkers Prev* **7:**1133-44 (1998)
3. Wang W, *et al. Cancer Res* **67:** 3505-11 (2007)
4. Goswami *et al. Cancer Res* **12:** 5278-83 (2005)

Acknowledgements

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