Metastatic and non-invasive breast cancer comparison study.

Patrick McQuaid
Center for Personalized Medicine, Center for Genetics & Pharmacology,
Roswell Park Cancer Institute 645 Virginia Street Buffalo NY

Abstract

My goal was to compare breast epithelial cells from two distinct cells lines using available cancer research tools and methods. One cell line was representative of non-tumorigenic tissue and the other of highly metastatic tissue. This experience has tangibly introduced me to a host of common lab techniques such as; cell culturing and maintenance, DNA/RNA extraction, PCR/qPCR, DNA sequencing and library prep.

I looked at three main dimensions in the cell lines;
1. Microscopic evaluation of cell morphology- metastatic cells displayed a more fibroblast-like appearance with long narrow projections. They also showed less cell-cell adhesion when viewed at similar cell concentrations.

2. Cell migration rate using transwells- highly metastaic cells showed slightly higher levels of migration through an 8 micron porous membrane after 18 hours incubation

3. Genetic analysis- DNA amplification with 4 sets of primers for erythroblastic leukemia viral oncogene homolog 3 (ERBB3 - a member of the epidermal growth factor receptor family of receptor tyrosine kinases) were sequenced in both cell lines. Analysis using CLUSTALW and BL2SEQ algorithms yielded no significant difference in nucleotide sequence across all 4 data sets.

Background

Breast cancer is the third most frequent cancer and affects approximately one in ten women in the western world. While it does account for nearly 40,000 annual deaths in this country, the survival rate among early detected tumors is quite high. It is only after the primary tumor has metastasized and spawned secondary tumors in the bone, liver, or lungs that it becomes fatal.
Cancer is understood to be the result of the accumulation of genetic alterations in a cell; including activation of oncogenes and inactivation of tumor suppressor genes. What are now described as the stages of a cancer represent the summative affects of these genetic changes. Certain genes, gene products, and gene expression levels have been found to correlate to different levels of tumor aggression. It is of interest then, to determine the genetic basis for the progression from non-invasive to metastatic conditions.
Cell migration plays an important role in the process of metastasis. The current approach of using transwells is one method to study cell migration as an analog for metastasis since the correlation between the *in vitro* migratory potential of tumor cells and their *in vivo* invasive properties was reported. Investigators can monitor genetic profiles alongside cell migration potential to determine possible relationships and evaluate therapeutics in less time and money than mouse models.

Materials and Methods

The cell lines analyzed were the immortalized human breast epithelial cell line MCF-10A, representing a non-tumorigenic state, and the human metastatic breast cell line MDA-MB-231, representing a malignant state. Existing cell cultures were subcultured just prior to reaching 80% confluency. Cells (200,000/coverslip) from each line were transferred to sterile coverslips and allowed to adhere during a 24 hour incubation period. These were then washed, stained, and mounted on slides for *morphological comparison*. For the *cell migration comparison*, cells were suspended in BD Falcon Cell Culture Inserts with a porous base membrane of 8 microns. Each cell line had 4 replicates in each condition. Conditions were set as in figure 1.

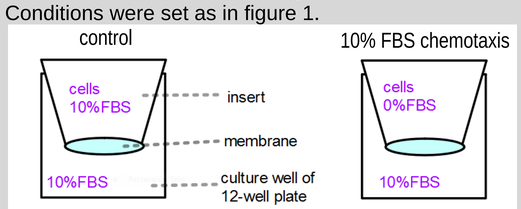


Figure 1. Cell migration experimental setup

DNA/RNA extraction from frozen pelleted cells was done using the Gentra Puregene Cell Kit and Qiagen miRNasy kit respectively. Extraction products were tested for quality using Agilent's bioanalyzer, Qubit fluorometer , and a Nanodrop spectrophotometer. PCR of select regions were amplified on a thermal cycler. PCR products were purified using the QIAquick 96 PCR Purification Kit Protocol and subsequently separated on agarose gel to confirm amplicon efficiency and correct size. Sanger sequencing was preformed on a ABI 3130xl Genetic Analyzer. qPCR was run on the ABI 7900HT SDS.

Results

Morphological Comparison

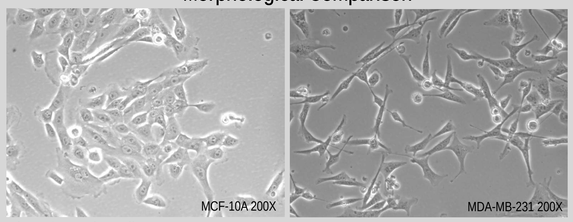


Figure 2. growing cells

Microscopic observation of growing and fixed/stained cell lines revealed less cell clumping, less spherical nuclei, and a more spindle-like appearance (like fibroblasts) in the metastatic MDA-MB-231 line.

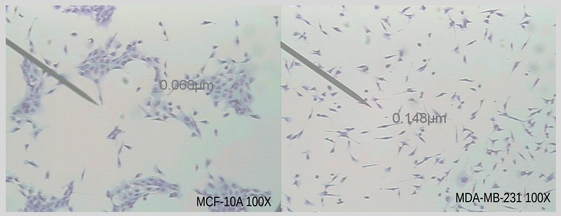


Figure 3. prepared slides stained with crystal violet

Results continued

Cell Migration

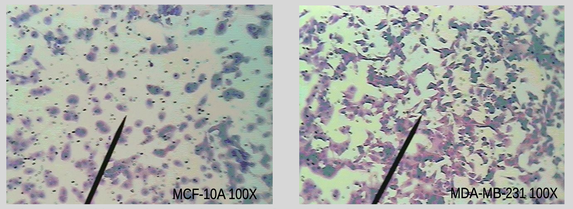


Figure 4. Scrubbed and removed membrane from culture inserts, stained crystal violet. This shows the cells that migrated through the 8-micron pored membrane

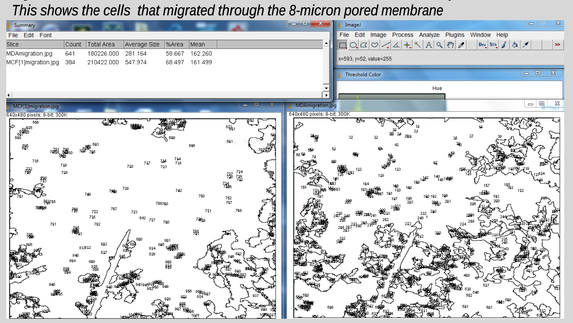


Figure 5. ImageJ analysis quantifying % free space. MDA cells showed a slightly higher propensity for migrating through the membrane, %coverage ~42% MDA to ~32%MCF

Genetic Analysis

All extraction product yields fell within industry norms for the given application. Specifically, RNA extraction yielded 110ng/ul in MCF and 62.1ng/ul in MDA via Qubit fluorometry. DNA extraction yield was 71.35ng/ul in MCF and 40.09ng/ul in MDA via nanodrop spectrophotometry. Amplicons from PCR reaction showed ~300bp fragments, as were expected from previous experiments with this primer set.

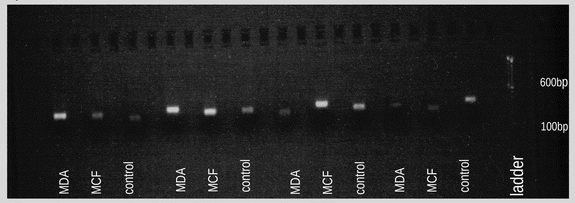


Figure 6. agarose gel verifying DNA amplicon from PCR

Sequence data was imported to Biology Workbench (http://workbench.sdsc.edu) and each of the 4 sets of sequences were aligned using CLUSTALW. All alignments showed nearly 100% conserved regions between MDA-MB-231 and MCF-10A.

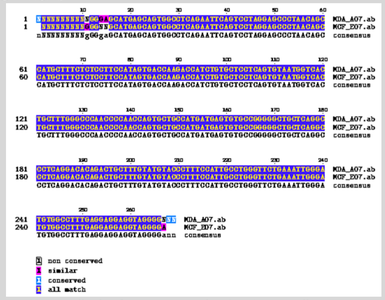


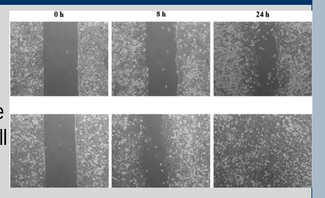
Figure 7. TEXSHADE display for representative CLUSTALW alignment

Conclusion

Differences in the metastatic breast cell line MDA-MB-231 and the non-tumorgenic line MCF-10A were observed regarding physical morphology and migration behavior across a porous membrane. Specifically, metastatic cells displayed less cell clumping and more spindle-like appearance. These cells also displayed slightly higher migration rates.
There was no difference found in the ERBB3 gene sequence using the 4 sets of primers available. The total length of this gene is reported as 5.7 kbp while our PCR products were all 250-300 bp so it is unclear whether any significant differences exist in the regions that were not sequenced. I suspect that most differences between these cells lines would appear at the transcript level and are best investigated using RNA and reverse transcriptional PCR assays.

Implementation

Students will be conducting a simpler wound healing assay to study rate of migration. Student designed experiments may include the use of chemo attractants or cell motility inhibitors like colchicine.



At this time I have not found a reasonably easy method to compare genomic DNA between the two cell lines. Restriction Landmark Genomic Scanning is a method for future study. Currently, students will make use of PCR and gel electrophoresis as they compare intron *Alu* elements within their own genomic DNA with the classroom population.

References

DeSantis, C., Siegel, R., Bandi, P. & Jemal, A. Breast cancer statistics, 2011. CA Cancer. J. Clin. 61, 409–418 (2011).
Klemke R.L., Leng J., Molander R., Brooks P.C., Vuori K., Cheresh D.A. (1998) CAS/Crk coupling serves as a "molecular switch" for induction of cell migration. J Cell Biol. Feb 23;140(4):961-72.
Molina, Angie, et al. "ATIP3, a novel prognostic marker of breast cancer patient survival, limits cancer cell migration and slows metastatic progression by regulating microtubule dynamics." *Cancer research* 73.9 (2013): 2905-2915.
The Physical Sciences-Oncology Centers Network. A physical sciences network characterization of non-tumorigenic andmetastatic cells. Sci. Rep. 3, 1449; DOI:10.1038/srep01449 (2013)
Subramaniam, S. (1998) The Biology Workbench--a seamless database and analysis environment for the biologist. Proteins, 32, 1-2.
Smith RA, Cokkinides V, Eyre HJ. Cancer screening in the United States, 2007: A review of current guidelines, practices, and prospects. CA. Cancer J. Clin. 2007;57:90–104.

Acknowledgements

Gratitude to the following;
Genomics Shared Resource RPCI
Jeffrey Conroy – co-director
Sean Glenn – co-director
Paul Quinn – research associate
Chris Borrelli – research technologist
Department of Cancer Genetics RPCI
Henry Withers – Pre-doctoral Candidate
National Science Foundation
Interdisciplinary Science and Engineering Partnership (ISEP)