Metastatic and non-invasive breast cancer comparison study.

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Abstract

My goal was to compare breast epithelial cells from two distinct cell 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 and non-invasive cell lines showed slightly higher levels of migration through an 8 micron porous membrane after 18 hours incubation. 2. Cell migration rate using transwells: highly metastatic cell lines showed increased migration in comparison to control conditions. 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) was sequenced in both cell lines. Analysis using CLUSTALW and BL2SEQ algorithms yielded no significant differences 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 to other parts of the body, such as the liver, or lungs that it becomes fatal.

Cell migration plays an important role in the process of metastasis. The current approach of using transwells is 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/coverlip) 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. What are now described as the stages of a cancer are: initiation, promotion, and progression, which reflect the progression from non-invasive to metastatic conditions.

Cell migration involves the ability of cells to move from one location to another, and it is a crucial aspect of tumor progression. The current approach of using transwells is 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.

Results continued

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. Amplification from PCR reaction showed ~300bp fragments, as expected from previous experiments with this primer set.

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

Conclusion

Differences in the metastatic breast cell line MDA-MB-231 and the non-tumorigenic cell 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.

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 elements within their own genomic DNA with the cancerous cells.

References


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