

Characterization of GAGE Antigen Expression in Thirteen Common Breast Cancer cell lines

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Introduction

- Breast Cancer Occurrence
- GAGE Antigens
- Expression Data of GAGE Antigens in Breast Cancer Cell Lines.



Why is Breast Cancer Research Important?

- 1 in 8 Women
- Estimates: 200,000 Women, 1,700 men Disagnosed
- Fatal: 40,000 Women and 450 Men
- In the US, 12% invasive



Breast Cancer Con't

- BRCA1 and BRCA2 genes = high susceptibility
 - 5%
- In 2006 the cost of breast cancer treatment in the US was estimated to be \$13.9 billion dollars.
- Further research may provide insight into breast cancer pathways involved in disease progression.



GAGE Antigens

- ▶ Chosen for investigation based on previous microarray results and subsequent investigation conducted at the University of North Dakota.
- ▶ Microarray data indicated that they were differentially regulated based on the presence or absence of the N- and C-terminal of MT-3.
- ▶ The goal of this research is to characterize the expression of GAGE family antigens across several ER receptor positive and negative breast cancer cell lines.



What are GAGE Antigens?

- ▶ Belong to a group of cancer/testis antigens normally expressed in human germline cells.
- ▶ Also found to be expressed in several tumor types.
- ▶ GAGE antigens fall into three groups.
- ▶ GAGE-1 has a unique C-terminal sequence no other member has.



GAGE Antigens Con't

- ▶ GAGEs 2 through 8 amino acid sequences are 98% identical.
- ▶ GAGE antigens may direct cell proliferation, differentiation, and the survival of germline cells.
- ▶ Normally expressed in adult male germ cells, and a subset of oocytes in the adult ovary.



GAGE Variants

- ▶ GAGE promoters lack a TATA box.
- ▶ Initiation can start at several different sites leading to transcripts of varying lengths.
- ▶ GAGE antigen family promoters only vary by two base pairs in the first 1400 base pairs.



GAGE Antigens and Cancer

- ▶ Present in 26% of breast cancers.
- ▶ Also expressed in stomach cancer, neuroblastoma, and esophageal cancer.
- ▶ Expression is correlated to a poor prognosis and aggressive tumor type.
- ▶ Normal expression is limited to immune privileged sites making them useful targets for prognostic indicators.
- ▶ Potential targets for immunotherapy.



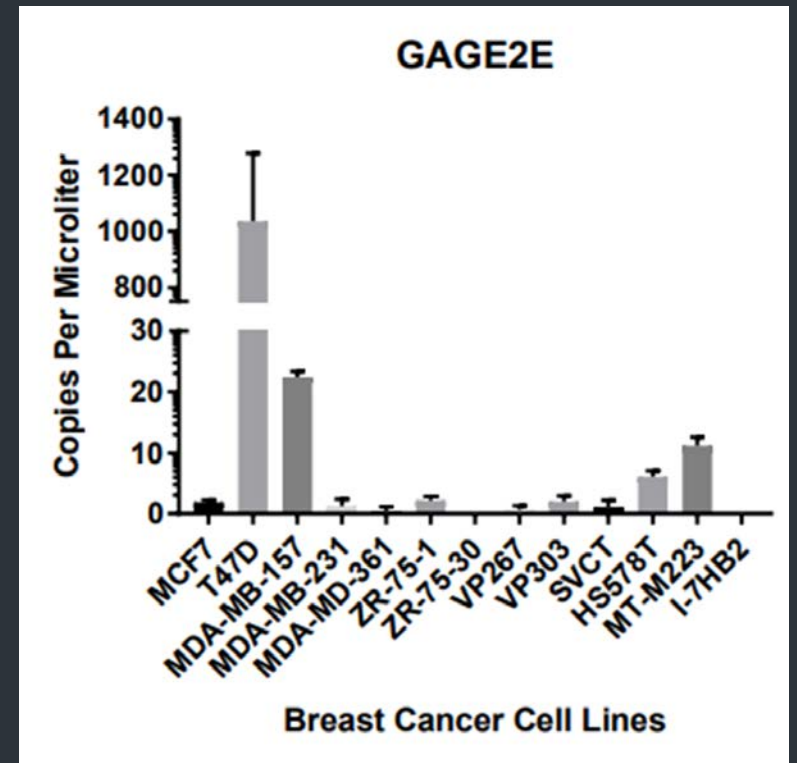
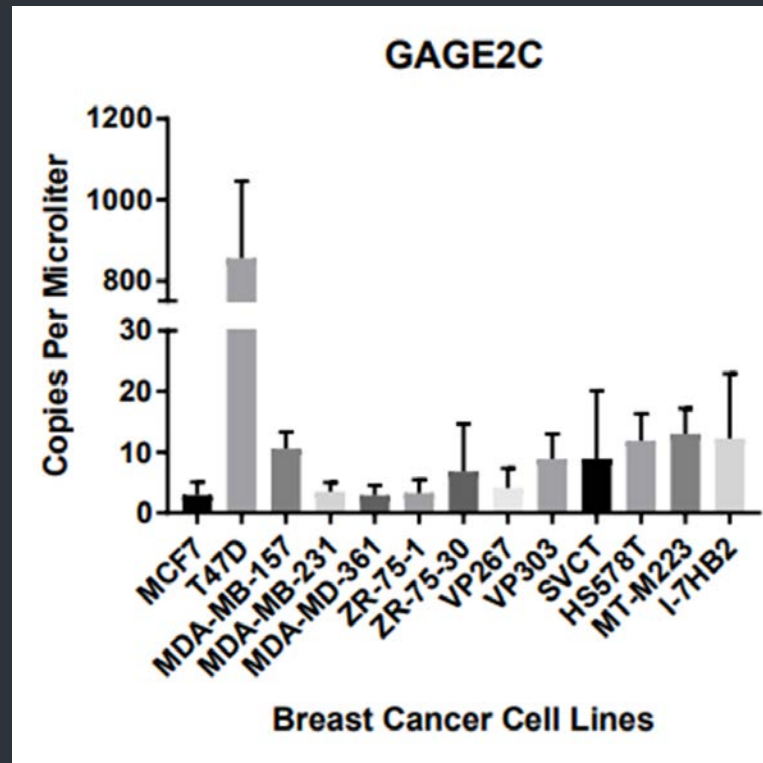
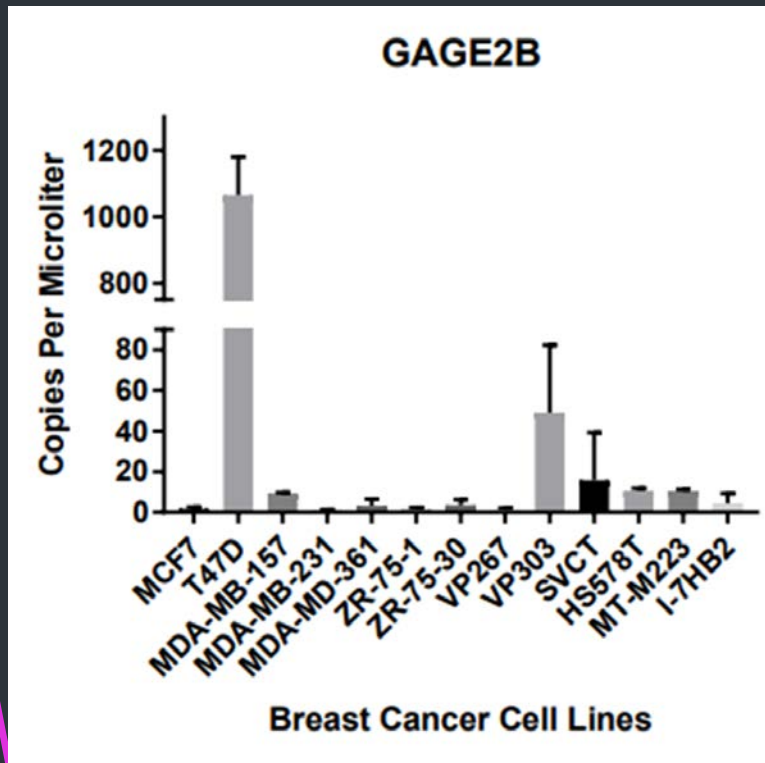
Breast Cancer Lines Characterized

- ▶ MCF7
- ▶ T47D
- ▶ MDA-MB-157
- ▶ MDA-MB-231
- ▶ MDA-MB-361
- ▶ 1-7HB2 Immortalized non-tumor forming cell line.



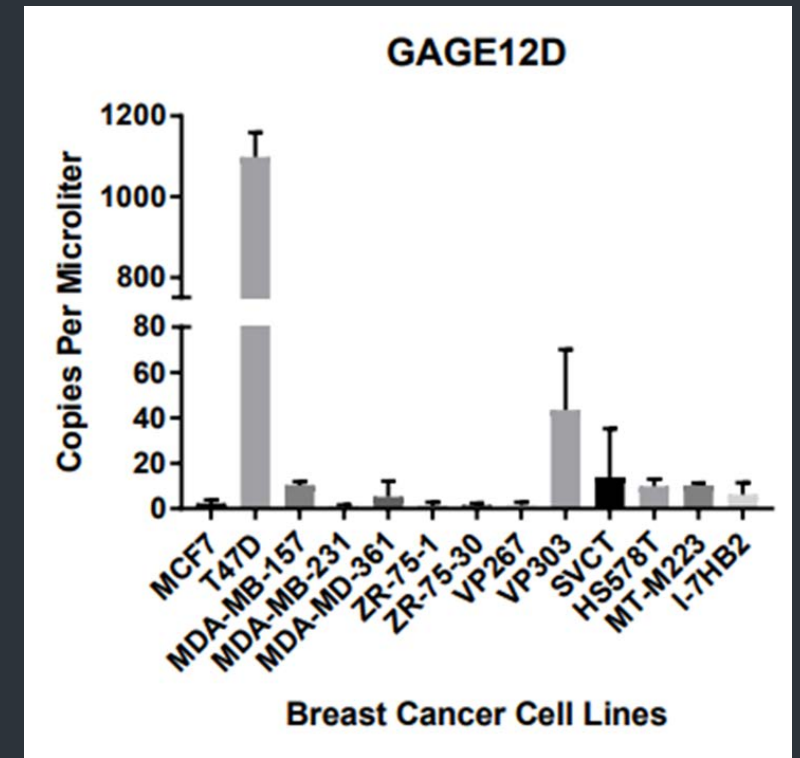
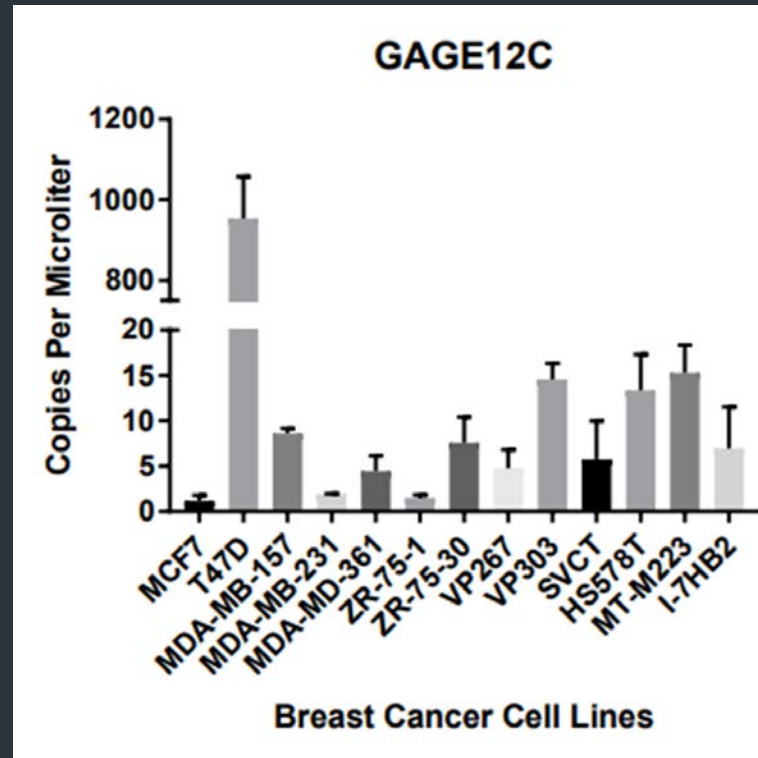
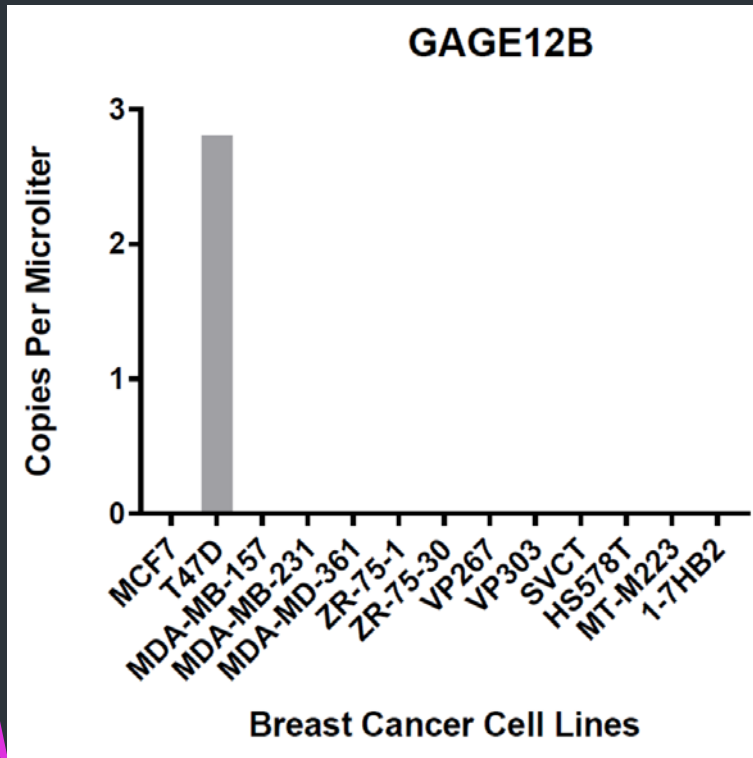
Breast Cancer Lines Characterized

- ▶ ZR-75-1
- ▶ ZR-75-30
- ▶ VP267
- ▶ VP303
- ▶ SVCT
- ▶ HS578T
- ▶ MT-M223



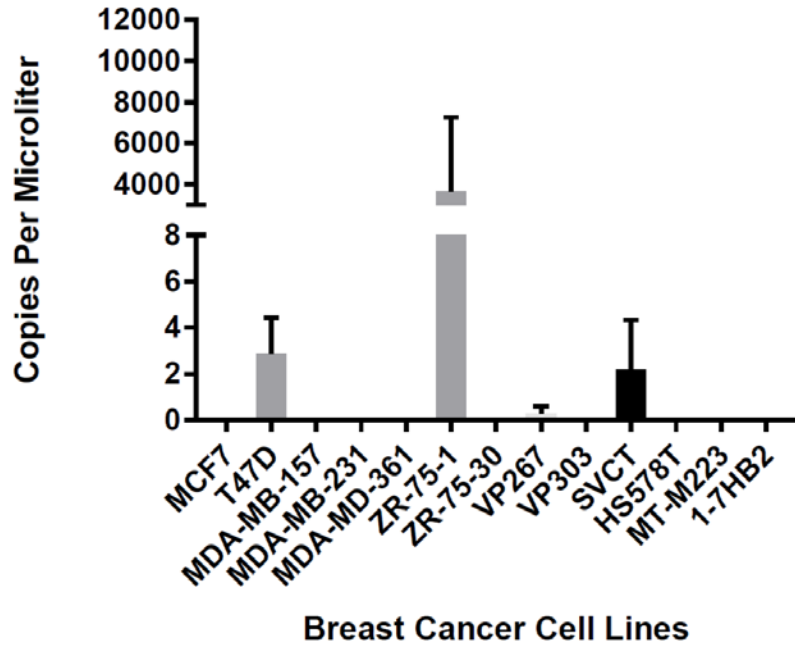
T47D cell line shows consistently high expression

Note: Statistics were not performed at this point. There is no single control group.

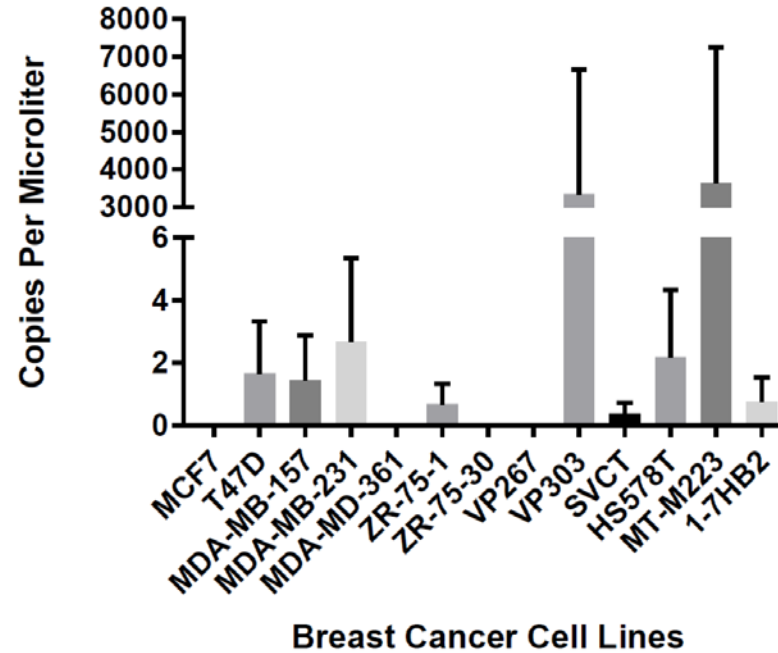


GAGE12B was only expressed in the T47D cell line. The expression was also lower than other GAGE antigen expression in T47D cell lines.

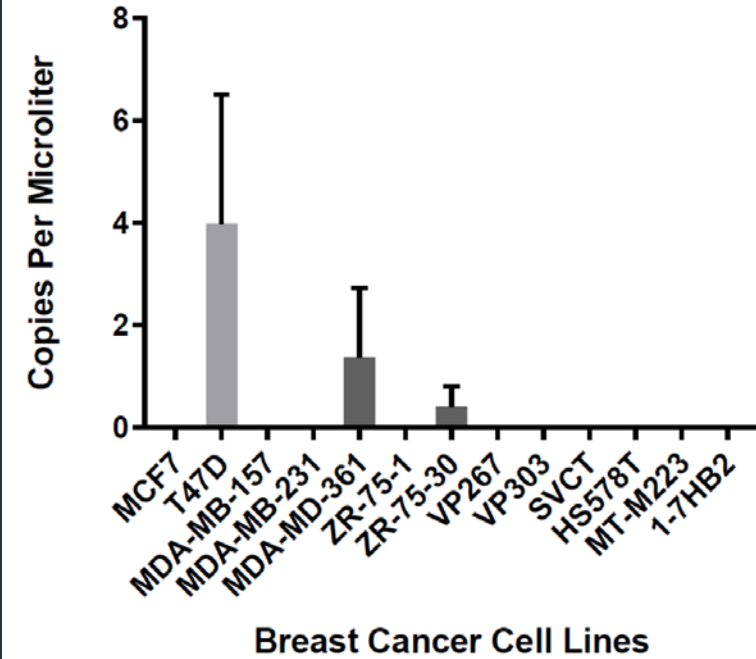
GAGE12J



GAGE13



GAGE06



GAGE12J has the highest expression levels in ZR-75-1 cells

GAGE13 has the highest expression levels in VP303 AND MT-M223 cells

GAGE06 is expressed at a very low copy number in T47D, MDA-MD-361 and ZR-75-01 cell lines.



Conclusions

- ▶ GAGE antigen expression is highly variable amongst the cell lines tested.
- ▶ There is no clear correlation between GAGE antigen expression and estrogen receptor status.
- ▶ Expression of GAGE2B, 2C, 12C, and 12D were highly expressed in T47D cells.



Future Directions

- ▶ Will determine expression of MT-1, MT-2, and MT-3 in the same cell lines as well.
- ▶ Goal is to determine if there is a correlation between metallothionein expression and GAGE antigen expression.
- ▶ Expression levels will be confirmed through western blotting.



Questions?





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