In vitro and In vivo Analysis of Breast Cancer Cell Lines

Alejandra E. Gallegos¹, Cynthia Annette Reyes², Paloma Valenzuela², Karla Parra², Natzielli Lerma², Courtney Becerri², Cynthia Rodriguez², and Giulio Francia²

Department of Biological Sciences, El Paso Community College¹
Department of Biological Sciences, University of Texas at El Paso²

Abstract

Our lab previously reported on the response of human Her-2 positive breast cancer cell grown in immunodeficient mice and treated with anti-Her-2 therapies. We also noted that there are a limited number of readily available human Her-2 positive breast cancer cell lines for preclinical studies, namely 231-G2N, B76 and MDA-MB-361. We consequently hypothesized that a larger repertoire of Her-2 breast cancer cell line would better reflect the different subtypes of human Her-2 positive breast cancers. To that end we began to study new Her-2 positive cell lines, including HCC1419 which was derived from the breast tumor of a Hispanic patient. We report our Western blotting analysis of protein expression levels of cancer associated genes (MLH1, MSH3, and Her-2) in breast cancer cell lines and in vivo evaluation of the growth of breast cancer models in mice. We also evaluated the in vitro growth of breast cancer cells as monolayers and as three-dimensional tumor spheroids. Thus BT474, MDA-MB-361, and HCC1419, formed highly cohesive spheroids when plated with theagarose overlay technique; in contrast, 231-G2N formed loose spheroids. By western blotting, we found that HCC1419 express high levels of Her-2 proteins relative to other breast cancer cell lines, using β-actin as a loading control. We were also able to detect the expression of proteins such as MLH1 and MSH3 and though their low levels of expression complicated analysis of the expression of the cells between monolayers and spheroids. MDA-MB-361 human Her-2 positive cell lines grow slowly in vivo (and in vitro, though they retain in vivo responsiveness to anti-Her-2 antibody therapy; e.g. p<0.5 for MDA-MB-361, n=5/group), which was increased by the addition of metronomic (20mg/kg/day) cyclophosphamide. We will present our data on the biological diversity of our library of preclinical models to study Her-2 positive breast cancer.

Results

Fig. 2a HCC1419 (HCC) spheroid formation over 4 days
Fig. 2b HCC1419 monolayer formation
Fig. 3 Western blots for MLH1 in breast cancer cells
Fig. 4 Western blots for Her-2 in breast cancer cells

Conclusion

HCC1419 has greater Her-2 protein overexpression than other breast cancer cell lines as indicated by Western-blot analysis. When Her-2 proteins are over expressed – termed Her-2 positive – breast cancer cells grow and divide uncontrollably. Our data indicates that HCC1419 breast cancer cells, isolated from the breast tumor of a Hispanic patient can form compact spheroid structures. We next plan to test the ability of HCC1419 to grow in mice, which would allow us to test new treatment strategies as we previously did with the MDA-MB361 tumor model. Our preliminary data has produced the foundation to conduct future experiment to identify new therapy regimens that will ultimately improve the quality of life in breast cancer patients.

Methods

Figure 1. a) Preparing a lysate sample from a pellet. b) Loading sample into the gel. c) Running the samples on the gel. d) Transfer of gel onto the membrane. e) Immunoblotting and Developing the membrane

Future Work

Fig. 5 MDA-MB561V3 were implanted orthotopically in SCID mice. Each group (n=5), was administered the following therapies: taxolubum (Tz, 20mg/kg/twice per week), CTX (20mg/kg/day), combination therapy (CTX+Tz) or Control (saline i.p/twice per week). The combination therapy significantly inhibited tumor growth compared to controls (p<0.05).

Fig. 6 Combination treatment using trastuzumab and metronomic chemotherapy. Trastuzumab antibody targets Her-2 positive breast cancer cells (red) and metronomic cyclophosphamide (CTX) targets the endothelial cell tumor vasculature (green)

References


Acknowledgements

Alejandra Gallegos and research reported in this publication was supported in part by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R25GM060424. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors would like to express special thanks to the staff of the Cytometry Screening and Imaging Core of the University of Texas at El Paso. This core facility is supported by the Research Centers in Minority Institutions (RCMI) program, Grant No. G12MD007592, to the Border Biomedical Research Center (BBRC) from the National Institutes on Minority Health and Health Disparities (NMHD), a component of the National Institutes of Health (NIH). A special thanks to the staff of the Genomic Analysis Core Facility of The University of Texas at El Paso. This core is supported by Grant G12MD007592 from the National Institutes on Minority Health and Health Disparities (NMHD), a component of the National Institutes of Health (NIH) and the Biomolecular Analysis Core Facility NIH Grant Number G12MD007592.