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14. ABSTRACT The basal-like subtype of breast cancer is both estrogen receptor and HER2 negative and therefore is not effectively treated by hormonal therapy or trastuzumab. The purpose of this research is to identify treatment options for this subset of breast cancer patients. Breast cell lines of basal-like and luminal origin were treated with five different chemotherapeutics to determine sensitivity levels. The basal-like cell lines were more sensitive to carboplatin than luminal lines. Next, we focused on identifying a biologic therapy targeting the basal-like subtype. HER1/EGFR is expressed in approximately 50% of the basal-like tumors while not expressed in the luminal tumors. The basal-like cell lines showed an increased sensitivity to HER1 tyrosine kinase inhibitor, gefitinib, compared to the luminal lines. Concurrent combinations of gefitinib and four chemotherapeutics indicate that most combinations are additive to synergistic. Of particular interest is carboplatin and gefitinib, which as single agents were more sensitive in the basal-like lines are also synergistic in combination. This work is support for a clinical trial at UNC, which will treat basal-like breast cancer patients with a HER1 inhibitor with or without carboplatin.					
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Introduction

Breast cancer is a heterogeneous disease. Both clinical data and gene expression data has suggested that there is more than just one type of breast cancer. Gene expression data identified five subtypes of breast cancer: the estrogen receptor positive Luminal A and B, HER2, normal-like and the basal-like(1). The basal-like subtype, which accounts for 10-15% of tumors, is negative for both estrogen receptor and HER2 and is therefore not a candidate for hormonal therapy or trastuzumab. In addition, most of the work with understanding chemotherapeutic sensitivity has been accomplished with the luminal subtype, which are more prevalent and have better outcomes. This prompted the work demonstrated here to identify chemotherapeutics with increased sensitivity and identify biologics that specifically target markers of the basal-like subtype. One candidate is the HER/EGF receptor, which is highly expressed in about 50% of breast cancer(2). Many inhibitors to this receptor are available and are in use in a variety of other cancers, decreasing the potential time to clinical use. Using cell line models, I hope to identify both chemotherapeutics and HER1 inhibitors that are effective in the basal-like subtype and identify a combination of drugs that may have maximum efficacy in clinical trials. This data will also be used to identify a gene expression profile that will identify which human patients have high expression of this pathway and hopefully would most likely benefit from this treatment.

Body

Task 1: Identify differences in toxicant sensitivity and gene expression profiles between basal and luminal breast derived cell lines treated with a diverse set of toxicants.

A cell line model was assembled with two immortalized human mammary epithelial cell (HMEC) lines of basal-like origin (ME16C, HME-CC), two tumor-derived basal-like lines (SUM102 and SUM149), and two tumor-derived luminal lines (MCF-7 and ZR-75-1). The panel of chemotherapeutics was determined by availability (or future availability) of tumor data treated with similar chemotherapeutics. Our panel included 5-fluorouracil (5FU), doxorubicin (DOX), carboplatin (Carbo), gemcitabine (GEM), and paclitaxel (PAC). Using MTT assays, we treated cells with a range of chemotherapeutics and determined the IC50 dose. There was a large amount of cell line variability across the different drug treatments; however, we were interested in a subtype specific response (Figure 1). The basal-like tumor derived lines, SUM102 and SUM149, were both more sensitive to carboplatin and slightly less sensitive to 5-fluorouracil than the HMECs and luminal lines.

Cell lines were treated with the IC50 dose of each chemotherapeutic and RNA was collected. DNA microarrays were performed. Microarray experiments were repeated until three arrays including a dye flip had high interclass correlations and clustered on the same dendrogram branch. For the basal-like experiments, this was relatively easy. However, for the luminal cell lines, there was a predominant dye flip problem that could not be resolved by using new reagents, arrays, and several biologic replicates. At this point we have just used the chemosensitivity data and are trying to find a way to use the data for the basal-like experiments. Once we figure out the dye flip problem or a way to use the current data, I will do more in depth

data analyses and comparisons with data from breast tumors treated with these chemotherapeutics.

Task 2. Evaluate the sensitivity of basal-like and luminal breast cell lines to HER1 inhibitors.

Using our panel of cell lines, we tested the sensitivity to the HER1 inhibitor gefitinib, a small molecule tyrosine kinase inhibitor. IC50 doses were determined after 72h of treatment with gefitinib (Figure 2). The basal-like lines were 2-10 fold more sensitive to gefitinib than the luminal tumors. This is consistent with the knowledge that the basal-like subtype expresses the receptor while the luminal subtype does not. I would like to also test erlotinib, an additional small molecule tyrosine kinase inhibitor, and cetuximab, a monoclonal antibody to the extra-cellular binding domain.

We designed an experiment to look at activation of the HER1 pathway. Cells were treated for 48h with gefitinib then the inhibitor was removed and fresh media was replaced. Time points were taken at 48h with inhibitor and 4h, 8h, and 24h after removal of the inhibitor. mRNA was isolated for use in microarray analysis. For the arrays, the 48h inhibitor sample was compared to untreated cells and the 4h, 8h, and 24h post inhibitor samples were compared to the 48h inhibitor sample. Microarrays have been completed for SUM102 and MCF-7. Preliminary analysis suggests that not only do the basal and luminal lines have differences in sensitivity, but they also have differences in their genomic response to gefitinib. Using an unsupervised approach, the samples clustered into three main groups – 48h gefitinib, 4h and 8h post, and 24h post. The SUM102 and MCF-7 experiments clustered together within each group but were on distinct branches within each group (Figure 3). The 48h gefitinib samples for both MCF-7 and SUM102 had a large set of genes that were down regulated in response to the inhibitor. The real difference between the luminal MCF-7 and basal-like SUM102 is in the 4 and 8 h post gefitinib experiments. These sets of genes most likely represent the EGFR signaling that is occurring before the induction of genes involved in proliferation that is up in both cell lines by 24h. In the basal-like SUM102 there is a large set of genes that are induced at these time points, while the luminal MCF-7 has relatively few gene expression changes occurring during these same time points. Further data analyses and addition of the other cell lines are still needed before more conclusions can be made. In addition, this data will also be analyzed using pathway analysis and westerns. I would also like to investigate some of the other HER1 inhibitors to make sure the effects that we are observing are related to the HER1 inhibition and not additional effects of the inhibitors.

Task 3. Test combination therapies of HER1 inhibitors and a diverse set of toxicants

Combinations of gefitinib and chemotherapeutics were examined for the tumor-derived cell lines – SUM102, SUM149, MCF-7, and ZR-75-1. Cells were treated concurrently with constant ratios of the IC50 doses of both gefitinib and chemotherapeutic over a range of doses and growth inhibition was measured by MTT assay. Doxorubicin, 5-fluorouracil, carboplatin, and paclitaxel were used in the combination studies (Figure 4). Drug interactions were assessed using CalcuSyn to determine the Combination Index (CI): CI values less than one indicate a synergistic response, CI values equal to one indicate an additive response, and CI values greater than one indicate an antagonistic response (Table 1)(3). Combinations with 5-fluorouracil were

synergistic in most cell lines and combinations with paclitaxel were mostly antagonistic. Combinations with doxorubicin were more variable across the cell lines. The antagonistic response with paclitaxel is most likely a result of the concurrent treatment of gefitinib and drug. One drug could arrest the cells in one phase of the cell cycle thus preventing the action of the second drug. Additional experiments examining schedule dependency would be useful. Of particular interest were the combinations with carboplatin. The basal-like lines were more sensitive to carboplatin as shown previously in task 1 and were more sensitive to gefitinib as shown in task 2. Therefore, the ability of these two drugs to demonstrate synergism when treated together would be extremely promising. The combination of carboplatin and gefitinib was synergistic in the basal-like lines but antagonistic in the luminal lines.

Further experiments that need to be done for this task include testing additional HER1 inhibitors cetuximab and potentially erlotinib for their combination interactions.

Key research accomplishments

- Basal-like tumor derived lines are more sensitive to carboplatin.
- Basal-like cell lines are sensitive to gefitinib
- Combination of carboplatin and gefitinib was synergistic in vitro

Reportable outcomes

- | | |
|-------------------|---|
| Abstracts/Posters | 1. Molecular Biology of Breast Cancer Meeting |
| | 2. Toxicogenomics Bi-Annual Meeting |
| | 3. European Breast Cancer Conference |

Conclusions

These experiments further demonstrate the differences between the basal-like and luminal subtypes of breast cancer. Sensitivity to a variety of chemotherapeutics in a panel of basal-like and luminal cell lines indicated a lot of cell line variation. When we focused on characteristics consistent within a subtype, we found that the basal-like tumor derived cell lines were more sensitive to carboplatin. BRCA1 plays a role in the repair of DNA damage due to platinum drugs by recruiting RAD51(4-6). Evidence from Sorlie et al suggests that BRCA1 mutations predispose patients to the basal-like subtype of breast cancer (7). A paper by Estrodt et al found that the SUM149 line has a deleterious mutation in BRCA1 and the SUM102 has a low level of BRCA1 transcript levels(8). The cell line information and the stratification of BRCA1 mutations in the basal-like tumors may be additional support that platinum drugs would be good candidate chemotherapies in the treatment of basal-like cancers.

HER1 has been identified as being upregulated in about 50% of basal-like breast cancers(2). In our panel of cell lines, the basal-like cells were more sensitive to the HER1 inhibitor gefitinib than the luminal lines. The combinations of gefitinib and chemotherapy were typically synergistic in the basal-like than luminal cell lines. This included the combination with carboplatin, which the basal-like lines were sensitive to and in combination with gefitinib were

synergistic. We are starting to use gene expression to determine the activation signature of the HER1 pathway in both basal-like and luminal cell lines.

This preliminary work is support for a clinical phase II trial at the University of North Carolina Hospitals. This trial will select for stage IV basal-like patients stratify into two groups – one group receives the HER1 inhibitor Cetuximab while the other group receives cetuximab plus carboplatin. If the cetuximab alone group acquires progressive disease they will be switched to the combination. My current research demonstrated that basal-like cell lines were sensitive to a HER1 inhibitor and to carboplatin and that the combination was synergistic. This clinical trial will help to determine if the results from the cell line experiments can be applied to basal-like breast tumor patients. The clinical trial will collect RNA before, during, and after treatment as well as response to therapy and outcome data. We will hopefully be able to use our cell line HER1 activation signatures with the response data from the patients to develop a gene signature that indicates an active HER1 pathway and can predict who may respond to treatment.

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7. T. Sørlie *et al.*, *Proc Natl Acad Sci U S A* **100**, 8418-23 (Jul 8, 2003).
8. F. Elstrodt *et al.*, *Cancer Res* **66**, 41-5 (Jan 1, 2006).

Appendices

1. Molecular Biology of Breast Cancer Meeting Abstract
2. Toxicogenomics Bi-Annual Meeting Abstract
3. European Breast Cancer Conference Abstract
4. CV

Molecular Biology of Breast Cancer Conference – Poster Abstract

Identification of drug targets for the treatment of Basal-like Tumors

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Genomic studies have identified at least five distinct subtypes of breast tumors. These subtypes are believed to develop from different epithelial cell types and show different overall survival outcomes. Of particular interest is the estrogen receptor (ER)-negative Basal-like subtype, which accounts for 10-15 percent of all breast tumors and shows poor outcomes. In the breast cancer clinic, there are currently two biologically directed therapies that target either the ER or HER2 proteins. The Basal-like tumors lack both of these proteins, and hence, the only treatment options for these patients are cytotoxic chemotherapies. Therefore, a goal of ours was to use primary breast tumor gene expression data and cell line models to identify and validate candidate biologically based therapies for Basal-like tumors.

To identify potential targets, the gene expression data for approximately 1500 drug targets was examined across a breast tumor data set of 150 samples. Squalene epoxidase (SQLE) was expressed in most Basal-like tumors, as well as in the Basal-like tumor-derived cell lines SUM102 and SUM149. SQLE is an attractive target because it is highly expressed, it is a rate-limiting step in the cholesterol biosynthetic pathway, and there is an available inhibitor (NB598). Recent studies using inhibitors of HMGCoA reductase (the first rate limiting step) in epithelial cell lines suggest that inhibition of this pathway may be a potential target for therapeutic intervention.

Using the SUM102 and SUM149 cell lines and two more widely used luminal/ER+ lines (MCF-7 and ZR-75-1), we treated cells with NB598 and separately with lovastatin (an HMGCoA reductase inhibitor) and determined their sensitivity by identifying their 72h IC₅₀ dose. Sensitivity was similar across three of the four cell lines for NB598, with the exception of SUM102, which was approximately 300X more sensitive. Conversely, sensitivity to lovastatin was similar across three of the four cell lines except MCF-7 was approximately 5X more resistant. Since many drugs are rarely used as single agents, we also looked at the interactions between these two inhibitors and commonly used chemotherapeutics. Drug combination sensitivities again varied across the four cell lines; however, it appears that combinations of NB598 and 5-fluorouracil were typically synergistic, while combinations with carboplatin or paclitaxel were typically antagonistic. Similar analyses are being performed for lovastatin/chemotherapy combinations. Gene expression responses of these cell lines were also assayed using DNA microarrays. The effect on the cholesterol pathway showed that for MCF-7 and SUM102, adding either inhibitor greatly induced most genes in the cholesterol biosynthetic pathway, while SUM149 treated with lovastatin showed induction of the pathway but treatment with NB598 did not. ZR-75-1 treated with either drug showed a slight reduction in expression of the pathway. These *in vitro* data suggest that inhibition of SQLE activity can reduce cell line proliferation rates and in some instances, was synergistic with chemotherapy. These data also suggest that inhibition of the cholesterol pathway by addition of HMGCoA reductase inhibitors is different than inhibition of the pathway with SQLE inhibitors.

Identification of New Therapies for Basal-Like Breast Tumors

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The basal-like subtype of human breast cancer is aggressive and shows poor patient outcomes. It also lacks expression of the estrogen receptor (ER) and HER2; therefore, cannot be treated with biological drugs that target these proteins. The only treatments for these patients are cytotoxic chemotherapy. Our goal is to develop a therapy for basal-like tumors that includes the use of a biologic agent and chemotherapy.

To identify potential biological targets, the gene expression data for ~1500 drug targets was examined across a breast tumor data set of 150 samples. HER1 and squalene epoxidase (SQLE) were identified as being highly expressed in basal-like tumors and cell lines. HER1 is a receptor tyrosine kinase that induces signaling cascades affecting growth, adhesion, differentiation, and apoptosis. Several inhibitors of HER1 exist that have promising efficacy in other cancers. SQLE is part of the cholesterol biosynthesis pathway, which is necessary for dividing cells. A specific squalene epoxidase inhibitor is available (NB-598), and we also have examined an HMG-CoA Reductase inhibitor (lovastatin), which targets the cholesterol pathway at an earlier step.

Using a panel of four cell lines that model the basal-like and luminal subtypes, we determined their relative sensitivities to a HER1 inhibitor (gefitinib) and to two cholesterol pathway inhibitors (lovastatin, NB-598). The basal-like cells were at least 2X more sensitive than the luminal cells to gefitinib. Since SQLE is also expressed in the luminal B tumors, cell line differences, but not subtype specific differences, in sensitivity were identified. Since most biological drugs are rarely used as single agents, we also looked at the interactions between these inhibitors and chemotherapeutics. Drug sensitivities for 5-fluorouracil, doxorubicin, carboplatin, and paclitaxel were determined. Our tumor-derived basal-like lines were more sensitive to carboplatin compared to the immortalized human mammary epithelial lines and the luminal tumor-derived lines. In general, biologics combined with 5-fluorouracil were typically synergistic, while combinations with paclitaxel were typically antagonistic. In addition, treatment with carboplatin and gefitinib were highly synergistic at low doses of each drug, suggesting a potential therapy for basal-like patients. These analyses should help to identify a combined biologic plus chemotherapy regimen that is efficacious for basal-like patients.

European Breast Cancer Conference – Poster Abstract

Identification of targeted therapies for basal-like breast tumors

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The basal-like subtype of human breast cancer is aggressive and shows poor patient outcomes. It also lacks expression of the estrogen receptor (ER) and HER2; therefore, cannot be treated with biological drugs that target these proteins. The only treatments for these patients are cytotoxic chemotherapy. Therapies are needed for basal-like tumors that include the use of a biologic agent and chemotherapy.

Analysis of gene expression for a set of drug targets across a breast tumor data set identified several potential targets for biologic agents. HER1 and squalene epoxidase (SQLE) were highly expressed in basal-like tumors and cell lines. HER1 is a receptor tyrosine kinase that induces signaling cascades affecting growth, adhesion, differentiation, and apoptosis. Several inhibitors of HER1 exist that have promising efficacy in other cancers. SQLE is part of the cholesterol biosynthesis pathway, which is necessary for dividing cells. A specific SQLE inhibitor is available (NB-598) and an HMG-CoA Reductase inhibitor (lovastatin) is available that targets the cholesterol pathway at an earlier step.

Using a panel of cell lines that model the basal-like and luminal subtypes, we determined their relative sensitivities to a HER1 inhibitor (gefitinib) and to two cholesterol pathway inhibitors (lovastatin, NB-598). The basal-like cells were at least two-fold more sensitive than the luminal cells to gefitinib. Although cell line differences were identified in response to cholesterol pathway inhibitors, subtype specific differences were not evident. The lack of a subtype-specific response may be partially explained by the fact that SQLE is also highly expressed in other breast cancer subtypes. Because biological drugs are rarely used alone, we also examined interactions between these inhibitors and the chemotherapeutics 5-fluorouracil, doxorubicin, carboplatin, and paclitaxel. Biologics combined with 5-fluorouracil were typically synergistic, while combinations with paclitaxel were typically antagonistic. Tumor-derived basal-like lines were more sensitive to carboplatin than immortalized human mammary epithelial lines or luminal tumor-derived lines. Moreover, carboplatin and gefitinib were highly synergistic at low doses in the basal-like subtype, suggesting a potential therapy for these tumors. These analyses identify combined biologic plus chemotherapy regimens that may be efficacious for basal-like patients.

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EDUCATION

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2001 **B.S. in Biology and B.A. in Chemistry**, West Virginia Wesleyan College, Buckhannon, WV

RESEARCH EXPERIENCE

Graduate Research Assistant, University of North Carolina at Chapel Hill, Curriculum in Genetics and Molecular Biology, August 2001 – Current.

Research Advisor: Charles M. Perou

- Studied chemotherapeutic and general stress responses of luminal and basal-like breast cancers using cell lines models and *in vivo* responses for each subtype.
- Employed cytotoxicity assays to assess phenotypic response to chemotherapeutics in breast cell lines.
- Gene expression studies of breast cell responses to cholesterol and HER1 inhibitors with specific emphasis on identifying potential therapies for basal-like breast cancer.
- Gene expression and cytotoxicity studies to examine how combinations of biologic agents and chemotherapeutics interact. Used statistical tools to assess synergy/antagonism due to co-treatment.
- Use of shRNA to knockdown TP53 and BRCA1 to establish their role in response to chemotherapy in the basal-like subtype.

Biological Science Aid, Appalachian Fruit Research Station, Agriculture Research Service, United States Department of Agriculture, Bardane, WV
1995-2001 (summers and part time during the academic year)

- Worked for a horticulturist, microbiologist, and plant physiologist
- Laboratory activities include assisting in carbohydrate analyses, preparation of culture media and plant tissues for *in vitro* culture, DNA and protein extractions from plant tissue, SEM preparation, biological pest control experiments, cloning and sequencing. Assist in care of experimental orchard, greenhouse crops, and washing glassware.

Biology Laboratory Assistant, West Virginia Wesleyan College, Buckhannon, WV, 1997-2001

- Assisted in biology laboratory preparations and *in vitro* culture media preparation.

TEACHING EXPERIENCE

Teaching Assistant for Genetic Analysis I, University of North Carolina at Chapel Hill, Curriculum in Genetics and Molecular Biology, Fall 2002, Fall 2003.

- Taught recitations and graded homework.

Teaching Assistant for Principles of Biology and Microbiology, West Virginia Wesleyan College, Buckhannon, WV, Biology Department, May 1999 – August 2001.

- Assisted with laboratory sessions.

PEER-REVIEWED PUBLICATIONS

Troester MA, **Hoadley KA***, Sørli T, Børresen-Dale AL, Lønning PE, Herbert BS, Shay JW, Kaufmann WK, and Perou CM. 2004. Cell-type specific responses to chemotherapeutics in breast cancer. *Cancer Research* 64: 4218-4226.

Troester MA, **Hoadley KA***, Parker JS, and Perou CM. 2004. Prediction of toxicant-specific gene expression signatures following chemotherapeutics treatment of breast cell lines. *Environmental Health Perspectives Toxicogenomics* 112(16): 1607-1613.

*Co-first author publications.

MANUSCRIPTS SUBMITTED

Troester MA, He X, **Hoadley KA**, Herschkowitz JI, Oh DS, and Perou CM. [Submitted to *Oncogene* April 2006] Gene expression changes associated with p53 status in breast cancer.

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HONORS/AWARDS

2005	Toxicogenomics Research Consortium Student Poster Award
2005	UNC Graduate Student Kenan Hobgood Award
2003	International Congress of Genetics Travel Scholarship
2003	University of North Carolina Graduate School Travel Scholarship
2000-2001	Phi Kappa Phi, National Honor Society
1998-2001	Beta Beta Beta, National Biology Honorary
1999, 2000	West Virginia Wesleyan Outstanding Biology Student
1998-2001	Mrs. Alice S. Hill Designated Scholar
1997-2001	West Virginia Wesleyan Honors Program
1997-2001	West Virginia Wesleyan Presidential Scholar

PRESENTATIONS

Hoadley KA and Perou CM. Identification of targeted therapies for basal-like breast tumors. European Breast Cancer Conference, Nice, France, March 21-25, 2006.

Hoadley KA and Perou CM. Identification of new therapies for basal-like breast tumors. National Institutes of Environmental Health Sciences Toxicogenomics Research Consortium Bi-Annual Meeting, Welches, OR, Dec 4-8, 2005.

Hoadley KA and Perou CM. Identification of drug targets for the treatment of basal-like tumors. Molecular Biology of Breast Cancer, Molde, Norway, June 21-26, 2005

Hoadley KA, Troester MA, and Perou CM. Tumor subtype specific chemotherapeutics responses in breast cancer. The Society of Toxicology, New Orleans, Louisiana, March 6-10, 2005

Hoadley KA, Troester MA, and Perou CM. Gene expression profiles of human breast cell lines treated with chemotherapeutics. The International Congress of Genetics, Melbourne, Australia, July 6-12, 2003

Supporting Data

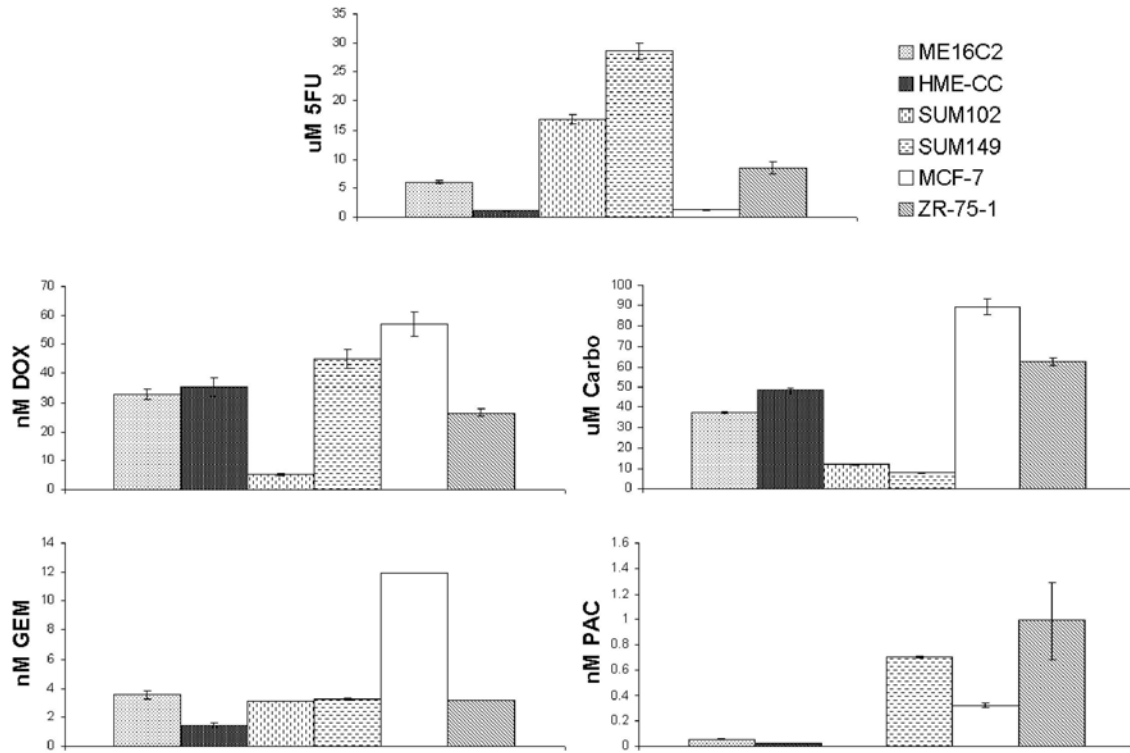


Figure 1. Comparison of chemotherapeutic inhibitory concentration values (IC₅₀) as determined by mitochondrial dye conversion assay (MTT) across the basal-like and luminal cell lines. IC₅₀ doses and the standard error are plotted for each chemotherapeutic.

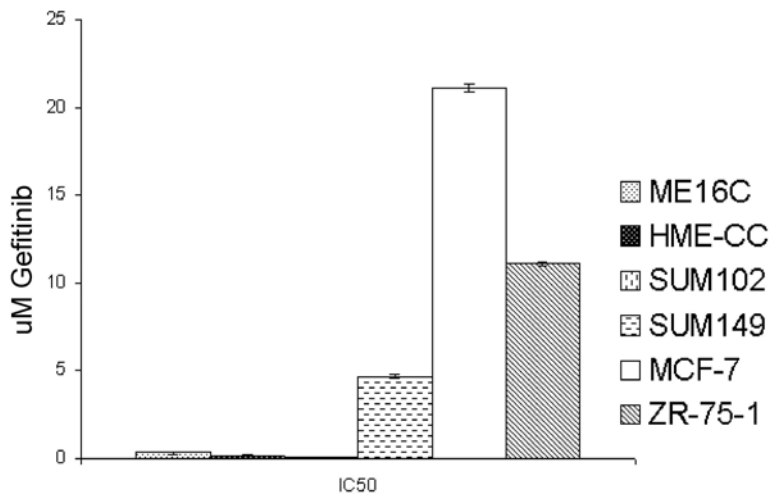


Figure 2. Comparison of gefitinib inhibitory concentration values (IC₅₀) as determined by mitochondrial dye conversion assay (MTT) across the basal-like and luminal cell lines. IC₅₀ doses and the standard error are plotted for each cell line.

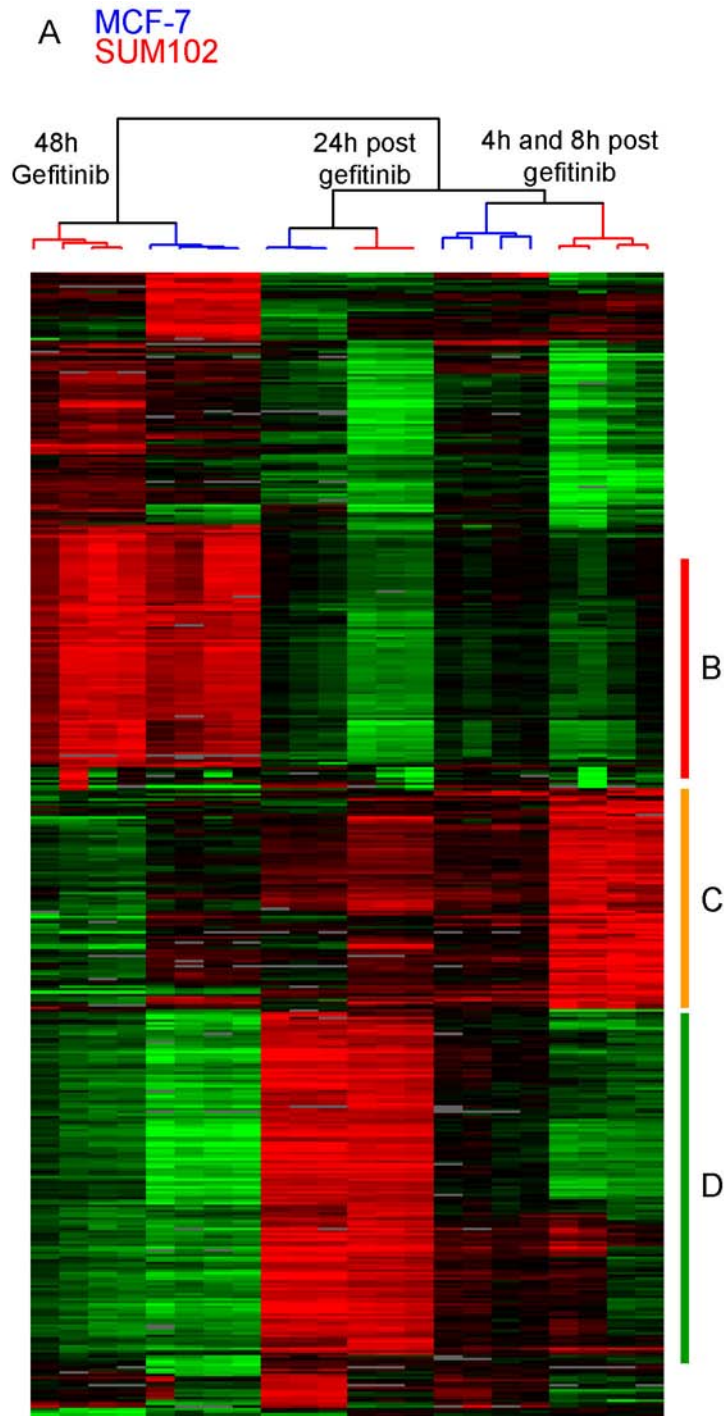


Figure 3. Genomic response to gefitinib in Basal-like SUM102 and luminal MCF-7 cells. Cells were treated for 48h with gefitinib followed by removal of the inhibitor, replacement of fresh media, and time points collected at 4h, 8h, and 24h post inhibitor treatment. A. Unsupervised analysis of MCF-7 and SUM102 experiments using genes with a 3-fold change in expression – 1025 genes. Experiments sorted by time point but each cell line was still on distinct branches within each time point group. B. A set of genes induced in both MCF-7 and ZR-75-1 gefitinib treatment. Most of these genes are not annotated. C. A set of genes up in the SUM102 4h and 8h post gefitinib treatment but not in the MCF-7, most likely representing the HER1 activation pathway in the basal-like cell line. D. Proliferation cluster induced by 24h in both the MCF-7 and SUM102 cells.

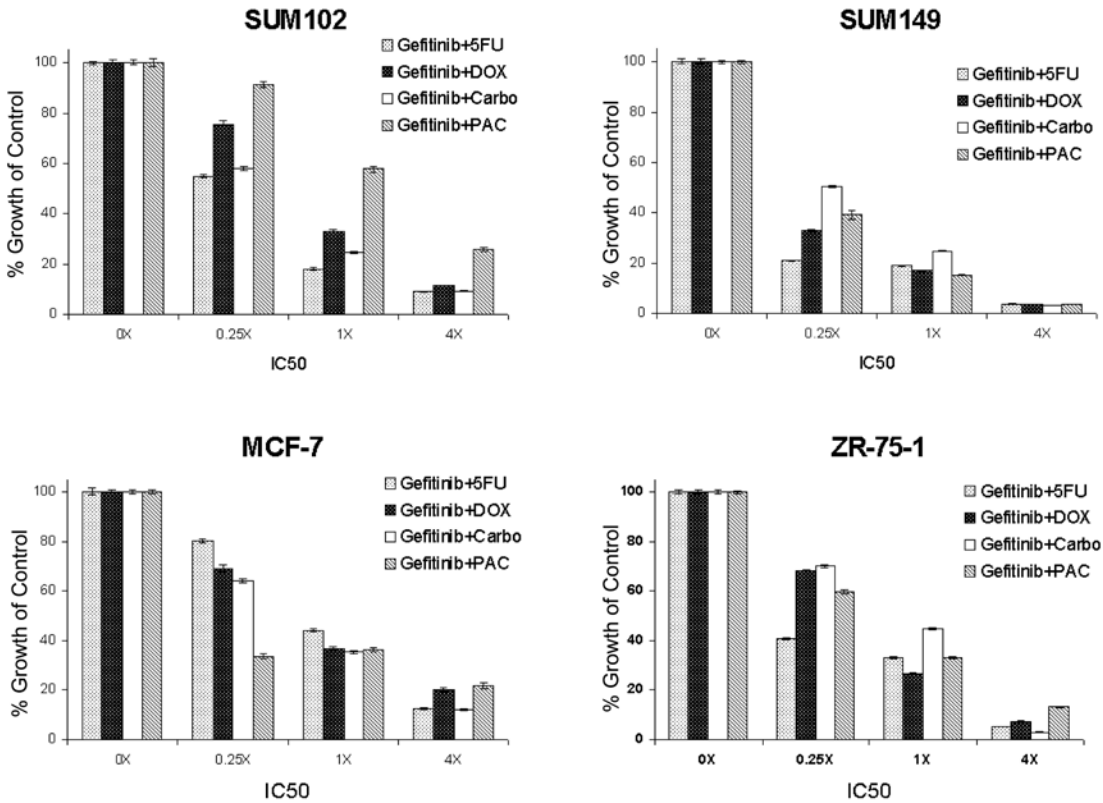


Figure 4. Gefitinib Combinations. Cells were treated for 72h with a constant ratio of the IC50 doses of gefitinib and chemotherapy at the IC50 dose (1X), at one-fourth that amount (0.25X) and four times that amount (4X). Values represent the mean percent growth of the control cells \pm SE.

Table 1. Combination Index (CI) of Basal-like and Luminal breast cell lines treated with a combination of gefitinib plus chemotherapy.

	Gefitinib Combinations			
	5FU	DOX	Carbo	PAC
SUM102	0.42	0.706	0.665	2.561
SUM149	0.42	0.866	0.921	0.758
MCF-7	1.057	1.23	1.395	1.036
ZR-75-1	0.69	0.653	1.629	0.601

CI values were determined by CalcuSyn software for doses of gefitinib and chemotherapy at the IC50 doses of each drug. A CI value equal to 1 indicates an additive response; a CI value less than 1 indicates a synergistic response; and a CI value greater than 1 indicates an antagonistic response.