AD_____

Award Number:

W81XWH-06-1-0452

TITLE:

 Crosstalk between leptin receptor and IGF-IR in breast cancer: a potential mediator of chemoresistance

PRINCIPAL INVESTIGATOR:

Rita Nahta, Ph.D.

CONTRACTING ORGANIZATION:

Emory University
Atlanta GA 30322

REPORT DATE:

April 2010

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
               Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

    × Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
01-04-2010	Annual	18 FEB 2009 - 19 MAR 2010

4. TITLE AND SUBTITLE
Crosstalk between leptin receptor and IGF-IR in breast cancer:

A potential mediator of chemoresistance

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0452

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Rita Nahta, Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Emory University

Atlanta, GA 30322

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel
Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Obesity is a major risk factor for breast cancer, and is associated with reduced treatment response and reduced overall survival. The obesity-associated hormones IGF-I and leptin and their receptors, IGF-IR and leptin receptor (Ob-R), are elevated in breast cancer. Previously we reported a novel interaction and cross-talk between IGF-IR and Ob-R in breast cancer cell lines. Our work this year has focused on determining the effects of combined inhibition of Ob-R and IGF-IR on breast cancer cell proliferation. Because we have been unable to identify an effective inhibitor of leptin receptor, we are using an inhibitor of JAK2, which is immediately downstream of the leptin receptor. Inhibition of JAK2 reduced MCF7 cell proliferation. Results described in this report indicate that adipocyte-secreted factors found in conditioned media collected from adipocytes may in fact alter response to taxanes. Furthermore, MCF7 cells appear to be dependent upon JAK2 signaling, which is downstream of leptin receptor and potentially upregulated during obesity. Thus, JAK2 inhibition downstream of leptin receptor is a potential strategy for combating obesity-associated breast cancer and possibly for improving chemosensitivity of obesity-associated breast cancers.

15. SUBJECT TERMS
Breast cancer, leptin, insulin-like growth factor-I, growth factor receptor signaling

| 16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	UU	9	19b. TELEPHONE NUMBER (include area code)

Table of Contents

INTRODUCTION

Obesity is an important risk factor associated with the development and progression of breast cancer (1-7), reduced therapeutic efficacy, and higher mortality rates among breast cancer patients (8-11). The obesity-associated hormones insulin-like growth factor-I (IGF-I) and leptin are found at high levels in breast cancer patients (12-15), and their receptors, IGF-IR and Ob-R (leptin receptor is also known as obesity receptor), are overexpressed in a majority of breast cancers (15-17). Increased expression of leptin and Ob-R correlate with increased risk for distant metastasis and reduced overall survival in breast cancer patients (15). Leptin induces proliferation of breast cancer cells via activation of STAT3 (18,19), a transcriptional activator of the anti-apoptotic protein Bcl-2 (20,21). STAT3-dependent overexpression of Bcl-2 was associated with resistance to the chemotherapeutic agent paclitaxel in breast cancer cells (21). We propose that IGF-IR and the leptin receptor interact, and that IGF-IR and leptin induce phosphorylation of Ob-R, activating STAT3 and upregulating Bcl-2, which in turn results in taxane resistance. Our hypothesis is that high levels of leptin and IGF-I increase Ob-R signaling, contributing to taxane resistance in breast cancer. Our long-term goal is to establish markers of Ob-R signaling as predictors of taxane response. The rationale is that these markers of Ob-R signaling, including serum levels of leptin and IGF-I, and tissue levels of phosphorylated Ob-R, STAT3 and Bcl-2, can be used (1) to identify patients most likely to respond to taxanes, and (2) as therapeutic targets to improve response rates to taxanes in the treatment of breast cancer.

Fig. 1. Dose-response to paclitaxel. MCF7 and MDA231 cells were treated with 2-fold serial dilutions of paclitaxel ranging from 5nM to 160nM for 6 days in 6 replicates per drug concentration. MTS assays were then performed and % proliferation is shown relative to control. Control reflects cells treated with the paclitaxel solvent DMSO at amount present in the highest concentration of paclitaxel (160 nM).

Fig. 2. Response of MCF7 cells to docetaxel in adipocyte conditioned media. MCF7 cells were plated in DMEM + 10% FCS (regular complete media), conditioned media (CM) from mouse 3T3 adipocytes, or CM from human abdominal omental cells (Om). Cells were untreated or treated for 6 days in 6 replicates per group with 10 nM docetaxel. MTS assays were performed and % proliferation is shown relative to DMSO control.

BODY

Task 1 Apply nanotechnology-based methods for visualization of IGF-IR and leptin receptor (Ob-R) in real time.

We are collaborating with Dr. Khalid Salaita in the Department of Chemistry at Emory University. As an initial experiment, Dr. Salaita used our BT474 cell line, which expresses high levels of IGF-IR and another receptor called HER2. He initially used an anti-HER2 antibody to test binding of fluorophore-conjugated antibodies to

4
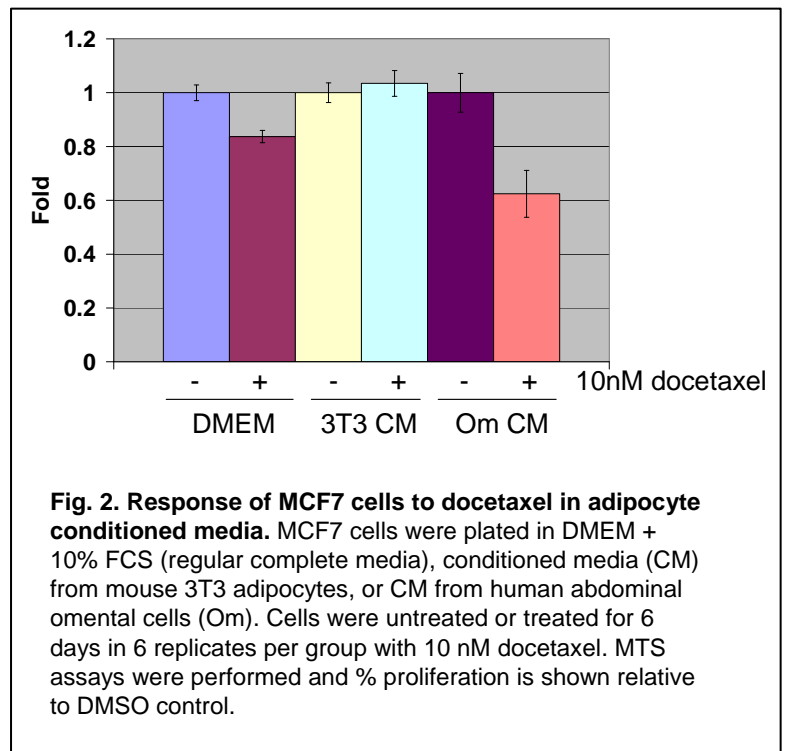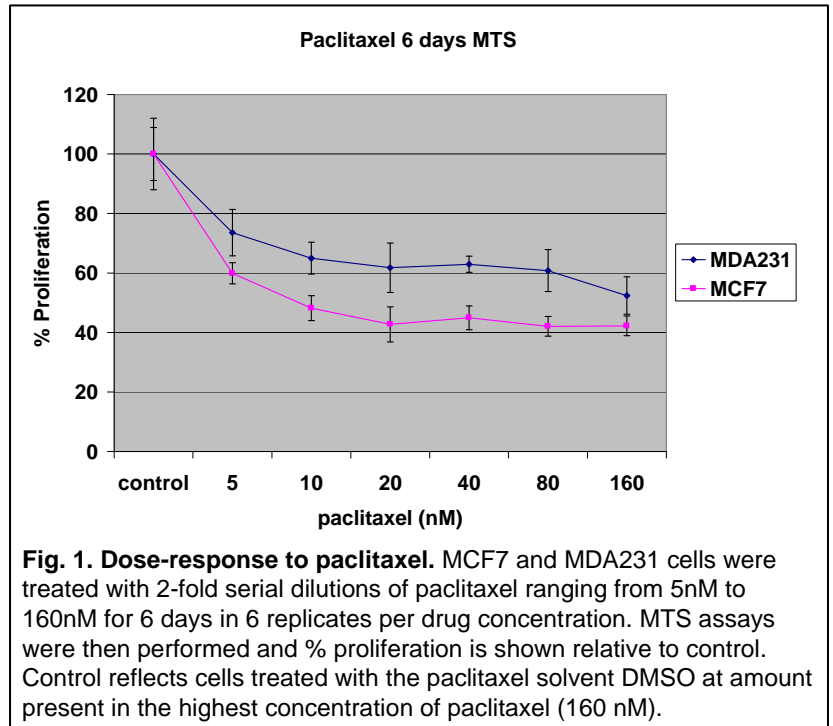
the membrane of these cells. Streptavidin-biotin labeling of the antibody was performed; cells showed efficient binding of the antibody to the cell surface by fluorescent microscopy. Thus, labeling was successfully achieved in this initial experiment. We are now able to collaborate with Dr. Salaita's group to label anti-IGF-IR antibody and anti-Ob-R antibody with streptavidin-biotin and expose MCF-7 and BT474 cells to these antibodies in the absence or presence of IGF-I or leptin. This aim was initially delayed due to difficulty in establishing a new collaboration at the PI's institution since the PI changed institutions in 2007. Now that the collaboration has been established and the method has been tested, we are in a position to complete the aim. A no-cost extension has been granted allowing us to complete the remainder of this aim and do additional experiments in aim 3 below.

Task 2   Demonstrate that IGF-I activates the leptin receptor via IGF-IR crosstalk.
Aim 2 has been completed and published (reference 22). Results from this publication were summarized in the 2009 annual report.

Task 3   Demonstrate that Ob-R signaling activated by leptin or IGF-I contributes to taxane resistance.
Dose-response profiles were established for MCF7 and MDA231 cells treated with paclitaxel (Fig. 1). The cell lines responded similarly to paclitaxel, with both lines showing 50% inhibition of proliferation (IC50) at approximately 10-20 nM. In our initial DoD application, we hypothesized that obesity-associated hormones will reduce taxane sensitivity. Thus, we treated MCF7 cells with DMSO control or 10 nM

Fig. 3. Dose-response to JAK2/STAT3 inhibitor WP1066. HCC1806, MDA231, and MDA468 cells were treated with 2-fold serial dilutions of WP1066 ranging from 1uM to 8uM for 6 days in 6 replicates per drug concentration. MTS assays were then performed and % proliferation is shown relative to DMSO control.

Fig. 4. Effect of combined inhibition of IGF-IR and JAK2 on MCF7 cell proliferation. MCF7 cells were treated for 6 days in 6 replicates per group with DMSO (C), IGF-IR inhibitor PPP ranging from 250nM to 1000nM, JAK2 inhibitor ranging from 5uM to 20uM, or a combination of the two inhibitors. MTS assays were performed and % proliferation is shown relative to DMSO control.

docetaxel while being maintained in regular cell culture DMEM media containing 10% fetal calf serum, conditioned media (CM) from 3T3 mouse adipocytes, or CM from human abdominal omental adipocytes. MTS proliferation assays were performed after 6 days (Fig. 2); all treatments were done in 6 replicates. Our results indicated that 3T3 CM reduced response to docetaxel, but that omental CM did not change response. Due to the conflicting results with 3T3 CM versus omental CM, this preliminary assay will be repeated in MCF7 cells and additional breast cancer cell lines to verify results. In addition, we hypothesized in the initial application that inhibition of Ob-R and IGF-IR signaling will increase taxane sensitivity. As reported in the previous annual report, we have been unable to validate siRNA or shRNA against Ob-R. Thus, we have used pharmacologic inhibition of downstream JAK/STAT signaling to determine if inhibition of Ob-R signaling affects taxane response. HCC1806, MDA231, and MDA468 breast cancer cells were treated with 2-fold serial dilutions of WP1066, a JAK2/STAT3 inhibitor, and proliferation was measured after 6 days (Fig. 3). All cells showed similar response with IC50 approaching 8uM. We will now treat cells with WP1066 plus docetaxel or paclitaxel to determine if WP1066 blocks inhibition of proliferation in response to taxanes. We have already treated MCF7 with the IGF-IR inhibitor PPP plus JAK2 inhibitor (Fig. 4), and have not observed any additive or
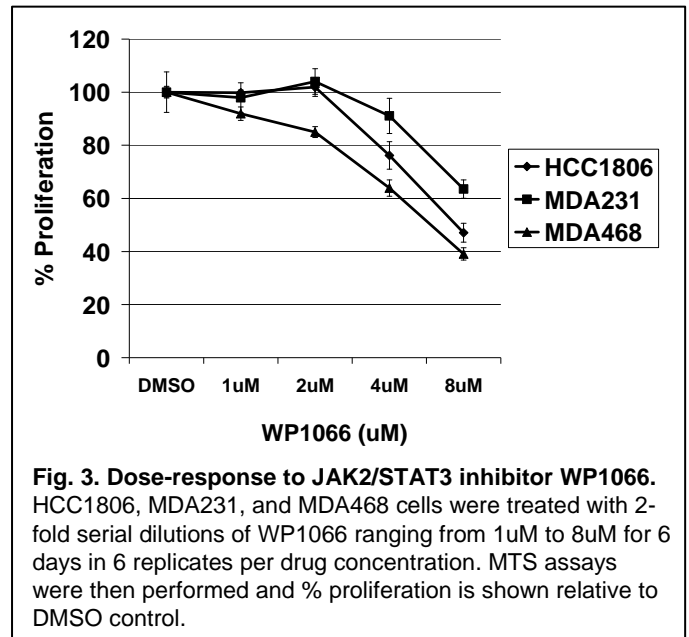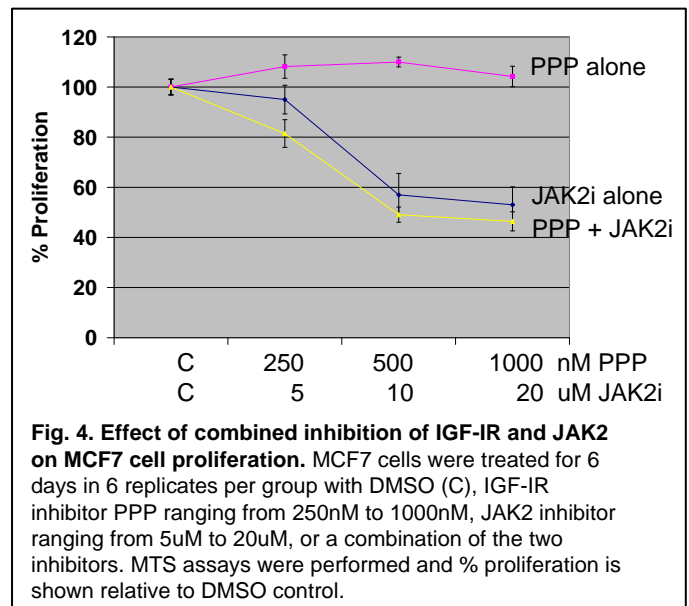
synergistic effects. Interestingly, despite having high endogenous levels of IGF-IR, MCF7 cell proliferation is unaffected by IGF-IR inhibition alone. In contrast, cells appear to be partially dependent upon JAK2 signaling, as JAK2 inhibition produced a dose-dependent decline in cell proliferation. The IC50 of JAK2 inhibitor in MCF7 cells was consistent with the IC50s observed in response to WP1066 in HCC1806, MDA231, and MDA468. Since cells appear to be sensitive to JAK2 inhibition, we will co-treat with docetaxel and JAK2 inhibitor to determine if there is synergy or additive effects. Based on our initial hypothesis, we expect that JAK2 inhibition will improve sensitivity to taxanes. Finally, since we have not found an effective siRNA against leptin receptor, we tested a mutant recombinant human leptin peptide. Mutant leptin did not increase inhibition of MCF7
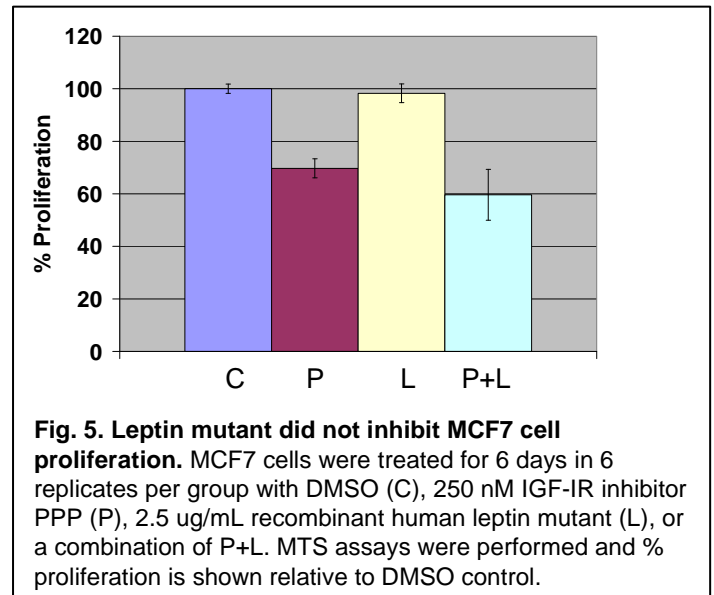

Fig. 5. Leptin mutant did not inhibit MCF7 cell proliferation. MCF7 cells were treated for 6 days in 6 replicates per group with DMSO (C), 250 nM IGF-IR inhibitor PPP (P), 2.5 ug/mL recombinant human leptin mutant (L), or a combination of P+L. MTS assays were performed and % proliferation is shown relative to DMSO control.

cell proliferation achieved by the IGF-IR inhibitor PPP (Fig. 5). These results are consistent with data shown in Fig. 4, indicating that PPP does not increase the effect of JAK2 inhibition on MCF7 cell proliferation. Interestingly, in Fig. 5, PPP alone did reduce proliferation, in contrast to Fig. 4. Thus, combined IGF-IR and Ob-R/JAK2 inhibition may not be beneficial, but rather JAK2 inhibition or IGF-IR inhibition alone may inhibit proliferation of breast cancer cells.

KEY RESEARCH ACCOMPLISHMENTS
During this past year, key accomplishments on this project are:
1. (1) The paclitaxel dose-response of a panel of breast cancer cell lines was determined.
2. (2) Initial results of adipocyte conditioned media on docetaxel dose-response demonstrated that adipocytes may reduce response to docetaxel but must be repeated.
3. (3) JAK2 inhibition produced a dose-dependent decline in MCF7 cell proliferation.
4. (4) Studies suggest that combined inhibition of IGF-IR and JAK2 may not be beneficial, but that single agent inhibition of IGF-IR or JAK2 may be effective. Future studies during this last year of no-cost extension will focus on the sensitizing effect of JAK2 or IGF-IR inhibition on taxanes.

REPORTABLE OUTCOMES
Receipt of this DoD Award as an indication of expertise in the area of breast cancer endocrinology and drug resistance has contributed to several opportunities for the PI. During the past year, the PI has been invited to serve on several grant review study sections:
1. (1) Department of Defense / CDMRP Breast Cancer Research Program (BCRP) Postdoctoral and IDEA Award Study Section #3 (IPA-3), 2009
2. (2) European Research Council, Reviewer for Advanced Grant Program - Physiology, Pathophysiology and Endocrinology (LS4) Panel, 2009
3. (3) New York State Peter T. Rowley Breast Cancer Research Project and Postdoctoral Fellowship peer review panel, 2010
4. (4) United States-Israel Binational Science Foundation, review panel member, 2010

In addition the PI has become an Editorial Board Member of the journal Breast Cancer: Basic and Clinical Research (3/2009-present).

CONCLUSION

As published in reference 22, we have made the following discoveries. (1) The IGF-I and leptin receptors interact in human breast cancer cells. (2) Cross signaling occurs from IGF-IR to Ob-R in breast cancer. IGF-I stimulation induces phosphorylation and activation of Ob-R, while IGF-IR kinase inhibition blocks IGF-I-mediated Ob-R activation. Downstream signaling molecules JAK2, STAT3, Akt, and ERK1/2, all of which are functional in the leptin and IGF-IR pathways as well as in multiple other signaling pathways, were activated by IGF-I stimulation. (3) Cross talk is unidirectional, as leptin does not activate IGF-IR. Thus, leptin is not likely to affect IGF-IR oncogenic function in breast cancer. However, since IGF-IR cross talks to Ob-R, it is feasible that Ob-R may contribute to IGF-IR molecular or biological effects, and is worthy of further study. Thus, we have identified a novel receptor interaction and unidirectional cross talk involving the IGF-IR and leptin receptor. As discussed in the previous annual report, we have also shown the following. (4) Insulin appears to stimulate Ob-R phosphorylation as well, although inhibition of insulin receptor does not block IGF-I-mediated Ob-R phosphorylation. Insulin is known to bind and activate IGF-IR; thus, insulin may be activating Ob-R via IGF-IR. (5) Our pilot study shows that IGF-I does partially reduce response to docetaxel. Results described in this report indicate that adipocyte-secreted factors found in conditioned media collected from adipocytes may in fact alter response to taxanes. Furthermore, MCF7 cells appear to be dependent upon JAK2 signaling, which is downstream of leptin receptor and potentially upregulated during obesity. Thus, JAK2 inhibition downstream of leptin receptor is a potential strategy for combating obesity-associated breast cancer and possibly for improving chemosensitivity of obesity-associated breast cancers.

REFERENCES

1. Morimoto LM, White E, Chen Z, Chlebowski RT, Hays J, Kuller L, Lopez AM, Manson J, Margolis KL, Muti PC, Stefanick ML, McTiernan A. (2002) Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). Cancer Causes Control 13: 741-751.

2. Sweeney C, Blair CK, Anderson KE, Lazovich D, Folsom AR. (2004) Risk factors for breast cancer in elderly women. Am J Epidemiol 160: 868-875.

3. Maehle BO, Tretli S, Thorsen T. (2004) The associations of obesity, lymph node status and prognosis in breast cancer patients: dependence on estrogen and progesterone receptor status. APMIS 112: 349-357.

4. Chlebowski RT. (2005) Obesity and early-stage breast cancer. J Clin Oncol 23: 1345-1347.

5. Camoriano JK, Loprinzi CL, Ingle JN, Therneau TM, Krook JE, Veeder MH. (1990) Weight change in women treated with adjuvant therapy or observed following mastectomy for node-positive breast cancer. J Clin Oncol 8: 1327-1334.

6. Irwin ML, McTiernan A, Baumgartner RN, Baumgartner KB, Bernstein L, Gilliland FD, Ballard-Barbash R. (2005) Changes in body fat and weight after a breast cancer diagnosis: influence of demographic, prognostic, and lifestyle factors. J Clin Oncol 23: 774-782.

7. Kroenke CH, Chen WY, Rosner B, Holmes MD. (2005) Weight, weight gain, and survival after breast cancer diagnosis. J Clin Oncol 23: 1370-1378.

8. Tormey DC, Gray R, Gilchrist K, Grage T, Carbone PP, Wolter J, Woll JE, Cummings FJ. (1990) Adjuvant chemohormonal therapy with cyclophosphamide, methotrexate, 5-fluorouracil, and prednisone (CMFP) or CMFP plus tamoxifen compared with CMF for premenopausal breast cancer patients. An Eastern Cooperative Oncology Group trial. Cancer 65: 200-206.

9. Bastarrachea J, Hortobagyi GN, Smith TL, Kau SW, Buzdar AU. (1994) Obesity as an adverse prognostic factor for patients receiving adjuvant chemotherapy for breast cancer. Ann Intern Med 120: 18-25.

10. Berclaz G, Li S, Price KN, Coates AS, Castiglione-Gertsch M, Rudenstam CM, Holmberg SB, Lindtner J, Erien D, Collins J, Snyder R, Thurlimann B, Fey MF, Mendiola C, Werner ID, Simoncini E, Crivellari D, Gelber RD, Goldhirsch A; International Breast Cancer Study Group. (2004) Body mass index as a prognostic feature in operable breast cancer: the International Breast Cancer Study Group experience. Ann Oncol 15: 875-884.

11. Enger SM, Greif JM, Polikoff J, Press M. (2004) Body weight correlates with mortality in early-stage breast cancer. Arch Surg 139: 954-958; discussion 958-960.

12. Schernhammer ES, Holly JM, Pollak MN, Hankinson SE. (2005) Circulating levels of insulin-like growth factors, their binding proteins, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 14: 699-704.

13. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M. (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 351: 1393-1396.

14. Tessitore L, Vizio B, Jenkins O, De Stefano I, Ritossa C, Argiles JM, Benedetto C, Mussa A. (2000) Leptin expression in colorectal and breast cancer patients. Int J Mol Med 5: 421-426.

15. Ishikawa M, Kitayama J, Nagawa H. (2004) Enhanced expression of leptin and leptin receptor (OB-R) in human breast cancer. Clin Cancer Res 10: 4325-4331.

16. Cullen KJ, Yee D, Sly WS, Perdue J, Hampton B, Lippman ME, Rosen N. (1990) Insulin-like growth factor receptor expression and function in human breast cancer. Cancer Res 50: 48-53.

17. Resnik JL, Reichart DB, Huey K, Webster NJ, Seely BL. (1998) Elevated insulin-like growth factor I receptor autophosphorylation and kinase activity in human breast cancer. Cancer Res 58:1159-1164.

18. Banks AS, Davis SM, Bates SH, Myers MG Jr. (2000) Activation of downstream signals by the long form of the leptin receptor. J Biol Chem 275:14563-14572.

19. Yin N, Wang D, Zhang H, Yi X, Sun X, Shi B, Wu H, Wu G, Wang X, Shang Y. (2004) Molecular mechanisms involved in the growth stimulation of breast cancer cells by leptin. Cancer Res 64: 5870-5875.

20. Stephanou A, Brar BK, Knight RA, Latchman DS. (2000) Opposing actions of STAT-1 and STAT-3 on the Bcl-2 and Bcl-x promoters. Cell Death Differ 7: 329-330.

21. Real PJ, Sierra A, De Juan A, Segovia JC, Lopez-Vega JM, Fernandez-Luna JL. (2002) Resistance to chemotherapy via Stat3-dependent overexpression of Bcl-2 in metastatic breast cancer cells. Oncogene 21: 7611-7618.

22. Ozbay T, Nahta R. (2008) A novel unidirectional cross talk from the insulin-like growth factor-I receptor to the leptin receptor in human breast cancer cells. Mol Cancer Res 6: 1052-1058.

APPENDICES
N/A

SUPPORTING DATA
N/A