Acquired Resistance to Tamoxifen Is Associated with Loss of the Type I Insulin-like Growth Factor Receptor: Implications for Breast Cancer Treatment

Dendra H. Fagan², Ryan R. Uselman¹, Deepali Sachdev¹,³, and Douglas Yee¹,²,³

Abstract

The role of the insulin-like growth factor (IGF) system in breast cancer is well defined, and inhibitors of this pathway are currently in clinical trials. The majority of anti-IGFIR clinical trials are in estrogen receptor–positive patients who have progressed on prior endocrine therapy; early reports show no benefit for addition of IGFIR inhibitors to endocrine therapy in this setting. In this study, we examined the effectiveness of IGFIR inhibitors in vitro by generating tamoxifen-resistant (TamR) cells. We found that TamR cells had diminished levels of IGF1R with unchanged levels of insulin receptor (IR), and failed to respond to IGF-I–induced Akt activation, proliferation, and anchorage-independent growth while retaining responsiveness to both insulin and IGF-II. The IGF1R antibody dalotuzumab inhibited IGF-I–mediated Akt phosphorylation, proliferation, and anchorage-independent growth in parental cells, but had no effect on TamR cells. An IGFIR tyrosine kinase inhibitor, AEW541, with equal potency for the IGFIR and IR, inhibited IGF-I–, IGF-II–, and insulin-stimulated Akt phosphorylation, proliferation, and anchorage-independent growth in parental cells. Interestingly, AEW541 also inhibited insulin- and IGF-II–stimulated effects in TamR cells. Tamoxifen-treated xenografts also had reduced levels of IGFIR, and dalotuzumab did not enhance the effect of tamoxifen. We conclude that cells selected for tamoxifen resistance in vitro have downregulated IGFIR making antibodies directed against this receptor ineffective. Inhibition of IR may be necessary to manage tamoxifen-resistant breast cancer.

Introduction

The first and arguably most effective targeted therapy for breast cancer involves inhibition of estrogen receptor (ER) function. Tamoxifen, a selective estrogen receptor modulator, has proven effective in both early and advanced stages of breast cancer (1). In addition, depriving receptors of ligand using aromatase inhibitors and degrading receptors through pure nonsteroidal anti-estrogens have also proven effective. Unfortunately, after initial success, a large portion of these tumors will develop resistance. This has led to the exploration and identification of additional targeted therapies, namely against growth factor receptors, such as EGFR, HER2, and IGF1R.

The IGFIR is a receptor tyrosine kinase that exerts its biologic effects through binding of the ligands IGF-I and IGF-II. Following, ligand binding and receptor activation, adaptor molecules are recruited, leading to activation of downstream pathways, including the mitogen-activated protein kinase (MAPK) and PI3K pathways, ultimately leading to proliferation, angiogenesis, resistance to apoptosis, and metastasis (2, 3). The closely related insulin receptor behaves in a similar manner, through its ligands insulin and IGF-II.

Cross-talk between the IGFIR and estrogen receptor has been well-documented and has led to clinical trials investigating the combined use of IGFIR and ER-inhibitors. Multiple studies have shown that ERα can enhance IGFIR signaling through transcriptional upregulation of IGFIR, IRS-1, and IGF-II (4–8). Reciprocally, IGFIR has been shown phosphorylate and activate ER on serine-167 through an S6-kinase mechanism (9). In addition to current IGFIR inhibitor clinical trials examining combined anti-IGFIR, anti-ER therapies, trials are also being conducted in endocrine-resistant populations.

The role of the IGFIR in cancer has been established and clinical trials evaluating inhibitors to this pathway are currently underway (10). As noted, preclinical studies have documented cross-talk between IGFIR and ER pathways (11), yet clinical trials conducted primarily in endocrine-resistant patients have been disappointing (12). In vitro and in vivo evaluation has been conducted using endocrine sensitive cells, with relatively little evidence showing the effectiveness of anti-IGFIR therapy in endocrine-resistant cells.

Two strategies of targeting the IGFIR are currently being evaluated in clinical trials. Monoclonal antibodies bind to the IGF1R, leading to receptor internalization and downregulation. Tyrosine kinase inhibitors bind to the ATP catalytic
domain of the internal tyrosine kinase domain of the IGF1R and the closely related insulin receptor. Although some view targeting of the IR dangerous because of metabolic consequences, recent data suggest a benefit to targeting the IR (13, 14). Multiple reports have showed a role for the insulin receptor in cancer biology (15–17). Furthermore, phase I clinical trials have shown limited metabolic consequences that can be treated using metformin (18). Thus, the clinical benefit of using IGF1R/IR tyrosine kinase inhibitors (TKI) may outweigh their potential metabolic side effects.

The overall aim of our study was to investigate the effectiveness of anti-IGF therapies using an endocrine resistant model. Herein, we reveal tamoxifen-resistant cells lack expression of IGF1R, and hence, are unaffected by IGF1R monoclonal antibodies. Tamoxifen-treated xenografts also have reduced levels of IGF1R and mice do not benefit from combined treatment with tamoxifen and dalotuzumab. Furthermore, complete and successful suppression of IGF1R signaling may require dual-inhibition of IGF1R and PI3K targets, as is currently under study in the clinic. Alternatively, endocrine-resistant patients may require the use of tyrosine kinase inhibitors, which are effective through inhibition of IR signaling.

Materials and Methods

Reagents
All chemical reagents were purchased from Sigma-Aldrich unless otherwise indicated. IGF-I, IGF-II, and insulin were purchased from Novozyymes GroPep Limited and Eli Lilly, respectively.

Cell lines and culture
All cells were grown at 37°C in a humidified atmosphere containing 5% CO₂ and supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin. MCF-7 cells were provided by C. Kent Osborne (Baylor College of Medicine) and maintained in improved MEM Richter’s modification medium (zinc option) supplemented with 5% FBS and 11.25 nmol/L insulin. MCF-7 TamR cells were generated by culturing MCF-7 in phenol-red free IMEM (zinc option) supplemented with 11.25 nmol/L insulin, 5% charcoal/dextran-treated FBS, and 100 nmol/L 4-OH tamoxifen. T47D cells were obtained from ATCC and maintained in MEM supplemented with 5% FBS and 6 ng/mL insulin. T47D TamR cells were generated by culturing T47D cells in phenol-red free IMEM supplemented with 5% charcoal/dextran-treated FBS, and 100 nmol/L 4-OH tamoxifen. TamR cells were grown in the presence of 4-OH tamoxifen for 6 months to allow resistance to develop before characterizing cells. As a control, parental cells were cultured for the same amount of time in regular media. Following the establishment of resistance, cells were passed for no more than 3 months.

Antibodies
Horseradish peroxidase–conjugated anti-phosphotyrosine (PY-20) was purchased from BD Biosciences. The ERα antibody used for Western blot analysis was purchased from Neomarkers Lab Vision. The IRβ antibody was purchased from Santa Cruz Biototechnology. Antibodies for phosphorylated Akt, IGF1Rβ, and total and phospho-p44/42 (MAPK/ERK) were purchased from Cell Signaling Technology. Anti-rabbit and anti-mouse horseradish peroxidase–conjugated secondary antibodies were purchased from Pierce.

Growth curve analysis
Cells were plated at a density of 1 × 10⁴ in 6-well plates and allowed to equilibrate overnight. Full medium was replaced with phenol-red free IMEM supplemented with 1% dextrancoated-charcoal (DCC)-FBS. 4-OH tamoxifen was added to cells at concentration and time as indicated in the figures. Cells were stained with trypan blue and counted using a hemacytometer.

Immunoblot
Cells were plated at a density of 3 × 10⁵ in 60-mm-diameter dishes and allowed to equilibrate overnight. Full medium was replaced with DCC-treated fetal calf serum for the next 3 to 5 days, after which all cells were switched to serum-free medium (SFM) for 24 hours. Upon reaching 70% confluence, cells were treated, placed on ice, washed twice with ice-cold PBS, and lysed with lysis buffer of 50 mmol/L Tris-Cl (pH 7.4), 1% Nonidet P-40, 2 mmol/L EDTA (pH 8.0), 100 mmol/L NaCl, 10 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonyl fluoride, 20 μg/mL leupeptin, and 20 μg/mL aprotinin. Lysates were clarified by centrifugation at 12,000 rpm for 15 minutes at 4°C. Protein concentrations were determined using the bicinchoninic acid protein assay reagent kit (Pierce). Cellular protein (50 μg) was separated by SDS-PAGE, transferred to a nitrocellulose membrane, and immunoblotted according to manufacturer guidelines.

Monolayer growth assay
Cells were plated in 24-well plates at a density of 30,000 cells per well, allowed to equilibrate overnight and starved in SFM media for 24 hours. After 5 days of treatment, growth was assessed via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described previously (19). Sixty microliters of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution in SFM was added to each well. After incubation for 3 hours at 37°C, wells were aspirated and formazan crystals were lysed with 500 μL of solubilization solution (95% DMSO + 5% MEM). Absorbance was measured with a plate reader at 570 nm using a 650 nm differential filter to assess growth.

Anchorage-independent growth
A 1-mL layer of 0.8% SeaPlaque-agarose (BioWhittaker) in 1% FBS-containing growth media was solidified into each well of a 6-well plate. The bottom layer was overlaid with 0.8 mL of a 0.45% top agar mixture for 10,000 cells per well with appropriate treatment. All plates were incubated at 37°C. After 12 days colony number was assessed on a light microscope with an ocular grid. Five random fields were counted per well and only colonies exceeding two thirds of a grid square were scored.
RNA isolation and quantitative real-time PCR

Cells were plated at a density of 1 × 10^4 in 100-mm-diameter dishes, allowed to equilibrate overnight, DCC starved for 3 days, and incubated overnight in SFM. Cells were treated with SFM or 1 nmol/L estradiol for 4 hours. Cellular RNA was isolated using TriPure Reagent according to the manufacturer (Roche). For quality control and to determine concentration, a 260:280 assay was conducted on a spectrophotometer. Forward and reverse primers were designed to target the following transcripts: PGR, KIAA0575, INSR, RPLD0 and IGF1R. A total of 2 μg of RNA was reverse transcribed using the Transcriptor Reverse Transcriptase Kit, and quantitative PCR was conducted using the Universal SYBR Green Kit according to the manufacturer's recommended protocol (Roche) on an Eppendorf Mastercycler Realplex4 machine. The relative concentration of mRNA was calculated using cycle threshold values that were derived from a standard curve and normalized to ribosomal protein, large, P0 as an internal control.

Xenograft growth

All animal protocols were approved by the University of Minnesota Institutional Animal Care and Use Committee. MCF-7L cells (5 × 10^6) were injected into the mammary fat pad of 5-week-old female ovariectomized athymic mice. One day before injection, mice were administered estrogen via drinking water at a concentration of 1 μmol/L as described previously (20). Tumors were allowed to achieve an average volume of 200 mm^3 before beginning treatment. Tamoxifen citrate (Sigma-Aldrich) was subcutaneously administered at a dose of 500 μg in a peanut oil emulsion daily for 5 of 7 days per week. Dalotuzumab was administered twice weekly via intra-peritoneal injection at a dose of 500 μg. Control animals were injected with histidine-based buffer and peanut oil alone. Tumor growth was measured bidirectionally and tumor volumes were calculated using the formula length × breadth^2/2.

Results

Tamoxifen-resistant cells are refractory to tamoxifen treatment but respond to estrogen treatment

To examine the effect of anti-IGF therapy in endocrine resistance, tamoxifen-resistant MCF-7L and T47D cells were generated. After selection, TamR cells survived in the presence of increasing concentrations of tamoxifen; however, parental cells were inhibited with as little as 1 nmol/L tamoxifen (Fig. 1A). Thus, TamR cells continued to survive and grow in the presence of tamoxifen, even up to concentrations of 1 μmol/L, showing resistance to the drug. Similar to some tamoxifen-resistant cancers, TamR cells maintained expression of estrogen receptor (Fig. 1B). Furthermore, TamR cells were able to proliferate in response to estrogen (Fig. 1C). When we examined gene expression regulated by ER in TamR cells, we found basal levels of estrogen regulated genes such as KIAA0575 (GREB1), PGR (Fig. 1D), TFF1, AREG, CTSD, and IGF1R (data not shown) were downregulated; however, estrogen was still able to stimulate transcription of these genes. Similar to the clinical situation of tamoxifen resistance where some tumors remain dependent on estradiol, our cells maintained estrogen receptor expression and responded to estrogen treatment.

Tamoxifen-resistant cells expressed low levels of IGF1R

Before examining the effectiveness of anti-IGF therapy in TamR cells, we examined the IGF signaling pathway and its components. Interestingly, IGF1R protein levels were diminished as measured by Western blot (Fig. 2A). Furthermore, TamR cells failed to phosphorylated Akt and MAPK after IGF-I treatment. The cells retained expression of IR and insulin and IGF-II ligand treatment resulted in phosphorylation of Akt and MAPK. To examine whether this change in IGF1R expression was because of decreased transcription, we conducted quantitative real-time PCR (qRT-PCR) to examine the message level of IGF1R. Indeed, IGF1R mRNA was decreased in TamR cells compared with parental cells (Fig. 2B). Treating TamR cells with estrogen resulted in a small increase in IGF1R mRNA, but did not restore the receptor to parental levels (Fig. 2B). Insulin receptor mRNA levels were not significantly different between parental and resistant cells (Fig. 2C). Furthermore, estrogen treatment did not affect IR levels in either cell line. These data show that tamoxifen-resistant cells lack IGF1R expression, but maintain expression of IR and are able to signal through IR.

Dalotuzumab inhibited signaling, proliferation, and anchorage-independent growth in parental, but not TamR cells

Dalotuzumab (MK-0646) is a humanized monoclonal antibody that binds the IGF1R. It has been shown to downregulate IGF1R in vitro and in vivo (21, 22). To examine the ability of the antibody to inhibit IGF-induced signaling, we pretreated MCF-7L parental and TamR cells with 20 μg/mL dalotuzumab for 24 hours before stimulating cells with ligand. Dalotuzumab inhibited IGF-I signaling, as measured via Akt and MAPK phosphorylation, in MCF-7L (Fig. 3A) and T47D (data not shown) parental cells and had a minimal effect on both insulin and IGF-II signaling. TamR cells did not respond to IGF-I, but Akt was activated by IGF-II and insulin. Dalotuzumab did not affect response to any of the ligands in TamR cells, presumably because of lack of IGF1R expression. To examine if this difference was also biologically relevant, we examined the effect of dalotuzumab on proliferation and anchorage-independent growth using the MTT and soft agar assays, respectively. All IGF system ligands tested induced proliferation in MCF-7L and T47D (data not shown) parental cells; however, only proliferation in response to IGF-I was inhibited in the presence of dalotuzumab (Fig. 3B). In contrast, insulin and to a lesser extent IGF-II stimulated the proliferation of TamR cells and this proliferation was not inhibited by dalotuzumab. Similarly, all ligands induced the anchorage-independent growth of MCF-7L parental cells (Fig. 3C) and dalotuzumab inhibited growth in response to IGF-I and IGF-II. In agreement with the signaling data, both insulin and IGF-II induced the anchorage-independent growth of TamR cells. This growth was not inhibited by dalotuzumab. Thus, dalotuzumab inhibited IGF-I induced signaling, proliferation, and anchorage-independent growth in MCF-7L parental cells, but had no...
effect in TamR cells, presumably because of their lack of IGF1R expression.

**AEW541 inhibited signaling, proliferation, and anchorage-independent growth in parental and TamR cells.**

AEW541 is a dual TKI that targets both IGF1R and insulin receptor. To examine the effect of IGF1R TKI's in endocrine resistance, we pretreated MCF-7L parental and TamR cells for 3 hours with 0.3 μmol/L AEW541 before stimulating cells with ligands. AEW541 inhibited insulin, IGF-I, and IGF-II signaling in MCF-7L cells (Fig. 4A) and T47D cells (data not shown). Furthermore, AEW541 was also able to inhibit insulin and IGF-II–stimulated phosphorylation of Akt and MAPK in TamR cells. To investigate whether this inhibition was also biologically important, we again examined...
proliferation and anchorage-independent growth. AEW541 was able to inhibit insulin, IGF-I, and IGF-II–stimulated proliferation in MCF-7L and T47D (data not shown) cells and insulin and IGF-II–stimulated proliferation in TamR cells (Fig. 4B). In addition, AEW541 was also able to inhibit insulin, IGF-I, and IGF-II–stimulated anchorage-independent growth in MCF-7L parental cells and insulin and IGF-II–stimulated anchorage-independent growth in TamR cells (Fig. 4C). Thus, AEW541 was able to inhibit signaling, proliferation, and anchorage-independent growth by suppressing both IGF1R and IR function in MCF-7L parental cells. Interestingly, AEW541 was also able to inhibit the growth of TamR cells presumably via suppression of IR signaling. These data show that TKIs, which target both IGF1R and IR, are effective in parental and resistant cells, because of inhibition of IR signaling.

**Dalotuzumab inhibited estrogen-stimulated growth but did not add to tamoxifen-mediated growth inhibition in vivo**

We next examined the effect of dalotuzumab on the in vivo growth of MCF-7L cells. Ovariectomized athymic mice were injected in the second mammary fat pad with MCF-7L cells as previously described (23). Mice were administered estrogen to stimulate tumor growth and tumors were allowed to establish (tumor volume of \( \sim 200 \) mm\(^3\)) before beginning treatment. Dalotuzumab (administered beginning at day 32) inhibited the growth of estrogen-stimulated tumors (Fig. 5A). To study the combination of tamoxifen and dalotuzumab, estradiol was withdrawn on day 32 and tamoxifen was started. Dalotuzumab treatment began simultaneously with tamoxifen (Tam + Dalotuzumab) or when tumors began to grow on tamoxifen alone (Tam → Dalotuzumab) at approximately day 74.
Tamoxifen-Resistant Cells Lose Expression of IGF1R

Tamoxifen by itself inhibited the growth of tumors; however, dalotuzumab coadministered with tamoxifen did not further suppress tumor growth. Furthermore, dalotuzumab did not significantly inhibit the growth of tamoxifen-resistant tumors when administered after the tumors began to grow on tamoxifen.

We next sought to determine whether this lack of efficacy of dalotuzumab in tamoxifen treatment was similar to the lack of IGF1R expression as observed in vitro. When tumors reached

Figure 3. Dalotuzumab can inhibit the growth of MCF-7L parental but not TamR cells. A, MCF-7L and TamR cells were serum starved overnight and pretreated with 20 μg/mL antibody for 24 hours before treating the cells with 10 nmol/L insulin, 5 nmol/L IGF-I, or 10 nmol/L IGF-II for 10 minutes. Cellular lysates were separated by SDS-PAGE, and levels of IGF1R, IR, phosphorylated Akt and MAPK, and total MAPK protein levels were assessed using specific antibodies by immunoblotting. B, MCF-7L and TamR cells were serum starved and treated with anti-IGF antibody along with ligand. Proliferation was evaluated using MTT assay, with results displayed as absorbance at 570 nm. Two-way ANOVA with Bonferroni comparison was used to compare the difference between antibody pretreatment and untreated samples; *, P < 0.01. C, MCF-7L and TamR cells were serum starved and treated with anti-IGF antibody and ligand in 1% FBS in 0.45% agar and overlaid on 0.8% bottom agar. Colony growth in agarose was assessed after 14 days. Colonies formed were counted and averaged from 5 individual microscopic fields. Results displayed are the average number of colonies in 5 fields of 3 wells. Two-way ANOVA with Bonferroni comparison was used to compare the difference between antibody pretreatment and untreated samples; *, P < 0.01.

Figure 4. AEW541 can inhibit the growth of MCF-7L and TamR cells. A, MCF-7L and TamR cells were serum starved overnight and pretreated with 0.3 μmol/L TKI for 3 hours before treating the cells with 10 nmol/L insulin, 5 nmol/L IGF-I, or 10 nmol/L IGF-II for 10 minutes. Cellular lysates were separated by SDS-PAGE, and levels of IGF1R, IR, phosphorylated Akt and MAPK, and total MAPK protein levels were assessed using specific antibodies by immunoblotting. B, MCF-7L and TamR cells were serum starved and treated with anti-IGF1R/IR TKI along with ligand. Proliferation was evaluated using MTT assay, with results displayed as absorbance at 570 nm. Two-way ANOVA with Bonferroni comparison was used to compare the difference between TKI treatment and untreated samples; *, P < 0.05; **, P < 0.005. C, MCF-7L and TamR cells were serum starved and treated with anti-IGF1R/IR TKI and ligand in 1% FBS in 0.45% agar and overlaid on 0.8% bottom agar. Colony growth in agarose was assessed after 14 days. Colonies formed were counted and averaged from 5 individual microscopic fields. Results displayed are the average number of colonies in 5 fields of 3 wells. Two-way ANOVA with Bonferroni comparison was used to compare the difference between TKI-treated and untreated samples; *, P < 0.01.
Discussion

The recently published results of IGF1R antibodies in clinical trials showing limited success in endocrine-resistant populations prompted us to investigate their efficacy using an endocrine-resistant model. Previous investigations into the efficacy of anti-IGF therapies have been conducted using endocrine-sensitive cell lines and xenograft models. Because IGF1R is an ER transcriptional target, understanding if IGF1R expression was affected by resistance to tamoxifen has clinical relevance. We found our tamoxifen-resistant cell lines lacked both protein and mRNA expression of IGF1R, but maintained expression of IR. This is in contrast to a report by Westley and colleagues showing that tamoxifen resistance in MCF-7 cells was associated with a dependence on IGF-I (24). This contrary finding may be a result of a difference in the way the tamoxifen-resistant cells were generated; these investigators used low serum conditions during generation of tamoxifen resistance while we used compete media. When cells were selected in this manner, tamoxifen became an ER agonist. Although seemingly contradictory, these findings are consistent with our own. IGF1R expression requires agonism of ER. In Westley and colleagues, their "tamoxifen resistant" cells tamoxifen-stimulated ER function. In our tamoxifen-resistant cells, we saw no evidence of agonistic activity stimulated by tamoxifen (Fig. 1D).

On the basis of prior reports of ER transcriptional regulation of IGF1R, it is not surprising that IGF1R expression would be decreased after acute treatment with a selective estrogen receptor modulator such as tamoxifen (25–27). Interestingly, studies conducted by Massarweh and colleagues using tamoxifen-resistant xenografts show decreased total levels of IGF1R, but basal phosphorylation of the receptor (28). This discordance may be explained by a difference in dosage of tamoxifen in model systems. In our model, tamoxifen is continuously administered to cells, whereas, in the Massarweh study, animals are given tamoxifen 5 times weekly, leading to the possibility that ER function is not completely suppressed in this model. Furthermore, this study did not clearly distinguish between tamoxifen-stimulated IR function. In our tamoxifen-resistant cells, we saw no evidence of agonistic activity stimulated by tamoxifen (Fig. 1D).

The finding that tamoxifen-resistant cells were refractory to IGF1R antibody treatment underscores the importance of using model systems similar to the patient populations the drug will be used in. Although several studies have showed the efficacy of IGF1R monoclonal antibodies in breast cancer cells, these cells have been endocrine sensitive (21, 23). The effect of combined anti-estrogen/anti-IGF1R treatment should also take into consideration whether the dose of anti-estrogen is sufficient, in and of itself, to suppress IGF1R function via receptor downregulation. Our in vivo results show that tamoxifen treatment results in decreased IGF1R mRNA levels. Initial results examining the effectiveness of IGF1R antibodies in endocrine-resistant breast cancer populations have not shown a definitive positive result (29, 30). This may be because of the lack of IGF1R expression in these patient populations. A recent study examining mRNA expression in a cohort of tamoxifen-resistant patients with breast cancer has showed a decrease in IGF1R levels in the recurrent tumors, suggesting our findings in vitro may correlate with the clinical scenario (31).
The efficacy of TKI’s in our tamoxifen-resistant cells underscores the importance of cotargeting the IR, along with the IGF1R. Initially, development of IGF1R inhibitors aimed to avoid targeting the IR, because of potential metabolic consequences. However, numerous studies by us and others have shown that the IR does indeed play a role in cancer biology (14, 18, 29, 32). Specifically, work by Hanahan and colleagues showed that IGF1R inhibition using antibodies is only successful in tumors/cells where the IGF1R/IR ratio is high. In addition, they show the IR can actually serve as an escape mechanism, providing resistance to IGF1R antibodies (17). Furthermore, work by Haluska and colleagues has shown that when fitigumumab (an IGF1R monoclonal antibody) is administered to patients, there is an associated increase in plasma insulin (33). This increase in insulin levels could potentially lead to increased IR signaling in tumor cells, providing yet another escape mechanism for the cancer cells to survive. These data are supported by a case report showing increased copy number of IR in a woman with metastatic hormone-refractory breast cancer (34). The role of the IR in cancer biology has been clearly defined, and the metabolic consequences of its inhibition are actively being investigated. A recent study conducted in mice showed that an IGF1R/IR TKI alone or in combination with tamoxifen did not lead to a significant change in glucose homeostasis, suggesting the drugs are tolerable. This study also showed the efficacy of the TKI in letrozole resistant xenografts, supporting our data that suggest TKI’s are more effective than antibodies in tamoxifen-resistant cells (35).

Although our data indicate IGF1R antibodies may not be effective in an endocrine-resistant system, this does not mean they have little use. Multiple trials are underway, examining the use of IGF1R antibodies in endocrine-sensitive populations. One promising area of investigation is inhibiting both the upstream (IGF1R) and downstream (mTOR) components of the IGF1R pathway, leading to maximal inhibition of signaling. Inhibition of IGF1R has been shown to sensitize cells to mTOR inhibition (36). In addition, clinical trials are underway examining the efficacy of combining IGF1R antibodies with mTOR inhibitors (37, 38).

Overall, our data highlight the importance of using model systems that will match the patient population the drug will ultimately be used in. In addition, when evaluating IGF1R therapies, it will be important to carefully select the proper patient population, as well as to verify the target is present. Finally, our data suggest dual IGF1R/IR TKI’s may be more effective than IGF1R antibodies, because of inhibition of IR. Combination therapy using IGF1R antibodies may require use of an mTOR inhibitor for complete suppression of the target, as is currently being evaluated in the clinic.

**Disclosure of Potential Conflicts of Interest**

D. Yee has received a commercial research grant from Merck. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

Conception and design: D.J.H. Fagan, D. Sachdev, D. Yee

Development of methodology: D.J.H. Fagan, D. Sachdev

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.J.H. Fagan, R.R. Uelmen, D. Sachdev

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.J.H. Fagan, D. Yee

Writing, review, and/or revision of the manuscript: D.J.H. Fagan, R.R. Uelmen, D. Sachdev, D. Yee

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Yee

Study supervision: D. Yee

**Grant Support**

This work was supported by Department of Defense Breast Cancer Research Program Pre-Doctoral fellowship grant B093938 (D.H. Fagan), Public Health Service grants CAT4285 (D. Yee), and Cancer Center Support Grant P30 CA7398, and support from the Merck Oncology Collaborative Studies program (D. Yee).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 22, 2012; revised April 30, 2012; accepted May 1, 2012; published OnlineFirst May 9, 2012.

**References**


Acquired Resistance to Tamoxifen Is Associated with Loss of the Type I Insulin-like Growth Factor Receptor: Implications for Breast Cancer Treatment


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-12-0684

Cited articles
This article cites 38 articles, 15 of which you can access for free at:
http://cancerres.aacrjournals.org/content/72/13/3372.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/72/13/3372.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.