A Kinome-Wide Screen Identifies the Insulin/IGF-I Receptor Pathway as a Mechanism of Escape from Hormone Dependence in Breast Cancer

Emily M. Fox1, Todd W. Miller2,6, Justin M. Balko1, Maria G. Kuba3, Violeta Sánchez1, R. Adam Smith5,7, Shuying Liu3,9, Ana María González-Angulo6,9, Gordon B. Mills9, Fei Ye4, Yu Shyr4,8, H. Charles Manning5,6,7, Elizabeth Buck10, and Carlos L. Arteaga1,2,6

Abstract

Estrogen receptor α (ER)–positive breast cancers adapt to hormone deprivation and become resistant to antiestrogens. In this study, we sought to identify kinases essential for growth of ER+ breast cancer cells resistant to long-term estrogen deprivation (LTED). A kinome-wide siRNA screen showed that the insulin receptor (InsR) is required for growth of MCF-7/LTED cells. Knockdown of InsR and/or insulin-like growth factor-I receptor (IGF-IR) inhibited growth of 3 of 4 LTED cell lines. Inhibition of InsR and IGF-IR with the dual tyrosine kinase inhibitor OSI-906 prevented the emergence of hormone-independent cells and tumors in vivo, inhibited parental and LTED cell growth and PI3K/AKT signaling, and suppressed growth of established MCF-7 xenografts in ovariectomized mice, whereas treatment with the neutralizing IGF-IR monoclonal antibody MAB391 was ineffective. Combined treatment with OSI-906 and the ER downregulator fulvestrant more effectively suppressed hormone-independent tumor growth than either drug alone. Finally, an insulin/IGF-I gene expression signature predicted recurrence-free survival in patients with ER+ breast cancer treated with the antiestrogen tamoxifen. We conclude that therapeutic targeting of both InsR and IGF-IR should be more effective than targeting IGF-IR alone in abrogating resistance to endocrine therapy in breast cancer. Cancer Res; 71(21); 1-–12. ©2011 AACR.

Introduction

Increasing evidence points to a role for insulin, insulin-like growth factor-I (IGF-I), and IGF-II in cancer development and progression (1, 2). The mitogenic actions of insulin are mediated by the insulin receptor (InsR) tyrosine kinase (2). Activated InsR phosphorylates InsR substrates (IRS-1 to -4), which bind the p85 subunit of phosphoinositide 3-kinase (PI3K). In turn, PI3K activates downstream effectors including AKT. InsR heterodimerizes with the highly homologous IGF-I receptor (IGF-IR), which also binds IGF-II and IGF-I (2).

Overexpression of InsR and IGF-IR has been detected in human breast cancers (3–5), and overexpression of either receptor is tumorigenic in mouse tumor models (6). Phosphorylated InsR/IGF-IR is present in all breast cancer subtypes, and high levels have been correlated with poor survival (7). IGF-IR has been pursued as a therapeutic target in cancer (8), but InsR has received less attention because of the potential for dysregulation of glucose homeostasis. Studies have implicated InsR in transformation and breast cancer mitogenesis, and hyperinsulinemia can accelerate mammary tumor progression in a mouse model of type II diabetes (9). Furthermore, type II diabetes and hyperinsulinemia are associated with increased breast cancer risk, and use of an inhaled form of insulin in patients with type I diabetes has been linked with breast cancer development (1).

Two-thirds of breast cancers express estrogen receptor α (ER) and/or progesterone receptor, biomarkers indicative of hormone dependence (10). Therapies for ER+ breast cancer inhibit ER function by antagonizing ligand binding to ER (tamoxifen), downregulating ER (fulvestrant), or blocking estrogen biosynthesis [aromatase inhibitors (AIs)]. However, many tumors exhibit de novo or acquired resistance to antiestrogens. One mechanism of resistance to endocrine therapy for which clinical data exist is overexpression of the ErbB2/HER2 protooncogene (11, 12). However, because less than 10% of ER+ breast cancers express high HER2 levels, mechanisms of escape from endocrine therapy remain to be discovered for most ER+ breast cancers. Using RNA interference (RNAi) screening and pharmacologic inhibitors of InsR and IGF-IR, we discovered InsR and IGF-IR are required for hormone-independent breast cancer cell growth, thus providing a...
targetable mechanism for breast cancers that escape estrogen deprivation.

Materials and Methods

Cell lines

Parental lines (ATCC) were maintained in improved minimum essential medium/10% FBS (Gibco) and authenticated by short tandem repeat profiling using Sanger sequencing (March 2011). Long-term estrogen deprivation (LTED) cells were generated in (13) and maintained in phenol red–free IMEM with 10% dextran/charcoal-treated FBS (DCC-FBS).

siRNA screen

MCF-7/LTED cells were transfected with the Dharmacon RTF Protein Kinase siRNA library (14) as in Supplementary Methods.

Cell proliferation

Cells in DCC-FBS with or without OSI-906 (OSI Pharmaceuticals), MAB391, IGF-I (R&D Systems), or insulin (Gibco) were counted or fixed/stained with crystal violet (13). For siRNA experiments, cells were transfected by using HiPerfect (Qiagen) and then reseeded and treated as earlier.

Immunoblot analysis and receptor tyrosine kinase arrays

Lysates from cells treated with OSI-906 or AEW541 (Selleck Chemicals) were subjected to immunoblot analysis (13). Immunoprecipitations were carried out by using Dynal protein G beads (Invitrogen), and 4G10 p-Tyr or p85 antibody (Millipore; ref. 15). Phospho–receptor tyrosine kinase (RTK) arrays were carried out by using the Human Phospho-RTK Array Kit according to manufacturer’s protocol (R&D Systems).

Mouse experiments

Mouse experiments were approved by the Vanderbilt Institutional Animal Care and Use Committee. Female ovariectomized athymic mice were implanted with a 14-day release 17β-estradiol (E2) pellet (0.17 mg) and 10⁷ MCF-7 cells. After more than 2 weeks, mice without palpable tumors (prevention experiment), or mice bearing tumors of 150 mm³ or more (treatment experiment) were randomized to vehicle (25 mmol/L tartaric acid), OSI-906 (50 mg/kg/day, per os), MAB391 (1 mg every 3 days, intraperitoneally), or fulvestrant (5 mg/wk, s.c.). Tumor volume in mm³ was measured 2 times a week by using the formula: volume = width² × length/2. Tumors were harvested and snap-frozen in liquid N₂ or fixed in 10% formalin prior to paraffin embedding for immunohistochemistry (IHC).

[¹⁸F]FDG-PET

2-deoxy-2-[¹⁸F]fluoro-d-glucose positron emission tomography ([¹⁸F]FDG-PET) was carried out as described (16).

Reverse-phase protein arrays

Core biopsies were obtained from patients with operable ER⁺/HER2-negative (HER2−) breast cancer treated with letrozole (2.5 mg/d) for 10 to 21 days. This study was approved by the Vanderbilt Institutional Review Board (VU-VICC-IRB-080064, NCT00651976). Tumor lysates were analyzed by reverse-phase protein arrays (RPPA; refs. 13, 17, 18).

Gene expression microarrays

MCF-7 cells were serum-starved for 24 hours and then treated with or without 10 µg/mL insulin for 4 or 24 hours. RNA was isolated and analyzed by gene expression microarrays.

Supplementary data

Additional details are provided in Supplementary Methods.

Results

RNAi screening implicates InsR in hormone-independent breast cancer cell growth

We previously established a panel of ER⁺ breast cancer cell lines selected after LTED (13). To identify kinases required for growth of these cells in the absence of hormones, we performed a high-throughput RNAi screen targeting 779 kinases. MCF-7/LTED cells were reverse-transfected with siRNA; cell viability was measured 4 days later (Supplementary Figs. S1 and S2). Median cell growth in 4 independent experiments was calculated for each siRNA. Individual knockdown of 42 kinases (Supplementary Table S1) inhibited MCF-7/LTED cell growth 33% or more (P < 0.05) in at least 3 of 4 experiments (Fig. 1A). Proteomic network analysis revealed that these 42 kinases map to several protein networks that overlap with InsR signaling, including PI3K (Supplementary Fig. S3). Knockdown of the InsR inhibited MCF-7/LTED growth by 35.2% compared with control siRNA (Fig. 1A). Because the InsR was a central node in the overlapping protein networks, and hyperactivation of the InsR/IGF-IR/PI3K/mTOR pathway has been implicated in acquired hormone-independent breast cancer cell growth (13), we selected InsR for further characterization.

We next quantified the expression of 190 total and phosphorylated proteins in surgical specimens from 10 patients with operable ER⁺/HER2− breast cancer that were treated for 10 to 21 days with the AI letrozole prior to surgery (Vanderbilt clinical trial NCT00651976). Tumor cell proliferation was assessed by Ki67 IHC in pre- and posttreatment biopsies. Of note, high Ki67 levels following short-term antiestrogen therapy have been associated with resistance to estrogen deprivation and poor patient outcome (19). By RPPA, the levels of 51 total and phospho-site–specific proteins correlated with the posttreatment Ki67 score (r > 0.4; Fig. 1B). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of these 51 proteins and phosphoproteins revealed that 13 were involved in insulin signaling (hsa04910) or were immediate effectors of this pathway (e.g., LKB1, S6; Fig. 1B; Supplementary Fig. S4). This represented a significant enrichment of insulin pathway members which correlated with the post-AI Ki67 (of the 190 antibodies screened by RPPA, 34 were involved in insulin signaling by KEGG; 1-sided x² test P = 0.049), further suggesting that InsR signaling is associated with adaptation of estrogen deprivation in human tumors.

Published OnlineFirst September 9, 2011; DOI: 10.1158/0008-5472.CAN-11-1295
Knockdown of InsR and IGF-IR inhibits hormone-independent growth and PI3K/AKT

Knockdown of InsR with an independent siRNA significantly inhibited growth of 3 of 4 LTED lines (Fig. 2A). Because InsR heterodimerizes with IGF-IR to activate PI3K, and RTK arrays revealed increased tyrosine phosphorylation of IGF-IR and/or InsR in 3 of 4 LTED lines (13), we also knocked down the IGF-IR. Knockdown of IGF-IR alone or in combination with InsR also inhibited growth of 3 of 4 LTED lines. However, the HER2-amplified MDA-361/LTED cell line was resistant to knockdown of both receptors (Fig. 2A). Receptor knockdown was confirmed by immunoblot (Supplementary Fig. S5). Knockdown of InsR or IGF-IR resulted in a compensatory upregulation of the other receptor (Fig. 2B), suggesting that combined knockdown would further inhibit signal transduction. Indeed, knockdown of either receptor reduced P-AKT in MCF-7 and MCF-7/LTED cells, but dual knockdown had an additive effect (Fig. 2B). In MCF-7/LTED cells, knockdown of InsR more effectively inhibited P-AKT than IGF-IR knockdown. Dual knockdown decreased P-AKT and P-S6 in ZR75-1/LTED and HCC-1428/LTED cells, as well as P-4EBP1 (an mTORC1 substrate) in ZR75-1/LTED cells (Fig. 2B), suggesting that both InsR and IGF-IR drive PI3K/AKT/TORC1 signaling and hormone-independent growth.

InsR/IGF-IR tyrosine kinase inhibitors block hormone-independent growth and suppress PI3K/AKT

We next examined the effects of the ATP-competitive dual InsR/IGF-IR tyrosine kinase inhibitors (TKI) OSI-906 (6, 20) and AEW541 (21). OSI-906 has shown antitumor activity against colorectal and non–small cell lung cancer xenografts (6, 16, 22). Treatment with both small molecules inhibited insulin- and IGF-I–induced phosphorylation of InsR, IGF-IR, and AKT (Fig. 3A). An approximate physiologic concentration of insulin in human plasma (60 pmol/L) did not activate PI3K/AKT (Fig. 3B). However, 10 μg/mL of insulin activated PI3K/AKT. This was blocked by knockdown of InsR but not IGF-IR, suggesting that, at this concentration, insulin acts mainly through the InsR. OSI-906 inhibited baseline InsR and IGF-IR phosphorylation in LTED cells (Supplementary Fig. S6). Notably, OSI-906 was more effective in the LTED derivatives of MCF-7 and MDA-361 cells compared with their parental counterparts (Fig. 3B). OSI-906 treatment disrupted the association between p85/PI3K and IRS-1 under steady-state and ligand-induced conditions (Fig. 3C), further suggesting that blockade of InsR/IGF-IR inhibits PI3K/AKT/TORC1 signaling.

We then assessed the effects of InsR/IGF-IR inhibition on growth of hormone-deprived cells. OSI-906 suppressed monolayer growth of 4 of 4 parental and LTED lines (Fig. 3D) and the anchorage-independent growth of 3 of 4 parental and LTED lines (Supplementary Fig. S7). Notably, OSI-906 was more effective in the LTED derivatives of MCF-7 and MDA-361 cells compared with their parental counterparts (Fig. 3D). To determine whether InsR/IGF-IR are required for the emergence of hormone-independent cells, parental cells were reselected in hormone-depleted medium. OSI-906 prevented or delayed the emergence of hormone-independent MCF-7, ZR75-1, HCC-1428, and MDA-361 cells (Supplementary Fig. S8), suggesting...
that InsR/IGF-IR signaling is required for adaptation to estrogen deprivation.

Inhibition of both InsR and IGF-IR prevents hormone-independent growth in vivo

MCF-7 xenografts were established in ovariectomized athymic female mice supplemented with a 14-day release E2 pellet. Upon expiration of the E2 pellet, the estrogen levels in these mice should mirror those found in postmenopausal patients treated with an AI. On day 15, mice without palpable tumors were randomized to treatment with vehicle or OSI-906. Six of 20 control mice (30%) developed tumors, whereas none of the 20 OSI-906-treated mice did (Fig. 4A).

We next assessed the effects of OSI-906 on established tumors. Following expiration of the E2 pellet, mice with tumors measuring 150 mm³ or more were randomized to treatment with vehicle or OSI-906. OSI-906 inhibited tumor growth compared with vehicle (Fig. 4B; *P < 0.05). To confirm drug target inhibition, tumors were harvested after 3 days of treatment. OSI-906-treated tumors exhibited markedly lower levels of phosphorylated IGF-IR, InsR, IRS-1, AKT, and S6 compared with vehicle controls (Fig. 4C). We also used [¹⁸F]FDG-PET as an indicator of OSI-906-mediated inhibition of PI3K/AKT (16). After 10 days of therapy, OSI-906 only modestly delayed xenograft growth (Supplementary Fig. S9). However, FDG uptake was significantly decreased 4 hours after a single dose of OSI-906 compared with baseline (Fig. 4D), suggestive of inactivation of PI3K/AKT in vivo. These data suggest that inhibition of InsR/IGF-IR with OSI-906 prevents the emergence of hormone-independent tumors and inhibits growth of established xenografts at least in part by inhibiting PI3K/AKT.

Blockade of InsR and IGF-IR is required to inhibit PI3K/AKT and prevent emergence of hormone-independent cells

We next asked whether the relevant therapeutic target of OSI-906 in LTED cells is the InsR, IGF-IR, or both. MAB391 is an IGF-IR–specific monoclonal antibody that binds the ectodomain of IGF-IR and downregulates IGF-IR homodimers and IGF-IR/InsR heterodimers (8). MAB391 downregulated IGF-I–induced activation of IGF-IR, modestly decreased IGF-I–induced P-AKT, but had little effect on insulin action. OSI-906 was more effective than MAB391 at
blocking IGF-I–induced P-AKT (Fig. 5A), as well as basal P-AKT and P-S6 levels (Fig. 5B). These data suggest that in LTED cells, InsR can maintain PI3K signaling in the absence of IGF-IR.

Treatment with OSI-906 but not MAB391 prevented or delayed the emergence of hormone-independent MCF-7, ZR75-1, HCC-1428, and MDA-361 cells following estrogen deprivation (Fig. 5C; Supplementary Fig. S8). Furthermore, OSI-906 treatment prevented the ability of insulin and IGF-I to rescue MCF-7 cells from estrogen deprivation, whereas MAB391 prevented rescue only by IGF-I but not by insulin (Fig. 5D; Supplementary Fig. S10). Notably, OSI-906 prevented rescue by IGF-I better than MAB391 (Fig. 5D), even though both drugs completely blocked IGF-IR phosphorylation (Fig. 5A). These results suggest that blockade of InsR and IGF-IR is required to prevent resistance to estrogen deprivation, and that inhibition of IGF-IR alone is insufficient.

**Dual InsR/IGF-IR blockade inhibits hormone-independent tumor growth**

We next compared the antitumor activity in vivo of OSI-906 and MAB391. Ovariectomized tumor-bearing mice were randomized to vehicle, OSI-906, MAB391, or the combination. OSI-906 significantly suppressed tumor growth compared with...
vehicle (Fig. 6A; \( P < 0.05 \)). Conversely, MAB391 had no effect even though it markedly downregulated tumor IGF-IR levels (Fig. 6B; Supplementary Fig. S11). The drug combination did not further suppress tumor growth compared with OSI-906 alone (Fig. 6A). OSI-906 but not MAB391 reduced levels of P-InsR/IGF-IR as measured by IHC (Fig. 6B; Supplementary Fig. S11). As shown with other IGF-IR antibodies and TKIs (23, 24), treatment with MAB391 and OSI-906 resulted in 1.4-fold and 50-fold increases in serum insulin levels, respectively (Fig. 6C); C-peptide levels were similarly altered (not shown). The antibody used for IHC detects both phosphorylated InsR and IGF-IR, thus implying that tumor cells maintain InsR activation when treated with an IGF-IR antibody. This suggests blockade of IGF-IR alone is insufficient to suppress hormone-independent tumor growth. Although we did not assess serum glucose levels, we should note that transient and reversible hyperglycemia has been observed in mice treated with OSI-906 (25).

Treatment with the ER downregulator fulvestrant has been shown to inhibit growth of MCF-7 cells and xenografts under estrogen-depleted conditions (26, 27). Others have shown that IGF-IR cross-talks with ER (28, 29), and fulvestrant reduces IGF-IR and IRS-2 expression in MCF-7 cells in estrogen-free conditions (30). Furthermore, therapeutic inhibition of InsR/IGF-IR in patients with endocrine-resistant breast cancer will likely be tested in combination with antiestrogens. Thus, we evaluated the combination of OSI-906 and fulvestrant on estrogen-independent MCF-7 xenograft growth. Ovariectomized mice with established tumors were randomized to vehicle, OSI-906, fulvestrant, or the combination. Both single-agent treatments inhibited tumor growth compared with vehicle (\( P < 0.05 \); Fig. 6D). The drug combination was superior to the single-agent treatments, inducing a complete tumor regression in 1 of 9 (11%) mice. This result suggests the simultaneous inhibition of ER and InsR/IGF-IR is more effective \textit{in vivo} against estrogen-deprived breast tumors.
Insulin/IGF-I–induced gene expression correlates with response to endocrine therapy

Herein, we carried out gene expression analysis to identify insulin-modulated pathways in ER\(^+\) breast cancer. MCF-7 cells were serum-starved for 24 hours followed by stimulation with insulin for 4 or 24 hours. RNA was isolated, and gene expression was analyzed by using microarrays. Notably, the signature consisting of genes whose expression levels changed after 4 or 24 hours of insulin treatment (\(P < 0.001\)) was inversely associated with recurrence-free survival (RFS) in 2 cohorts of patients with ER\(^+\) breast cancer treated with adjuvant tamoxifen for 5 years (MDACC298, \(n = 298\), \(P = 0.019\); LOI164, \(n = 164\), \(P = 0.074\); Fig. 7A; refs. 31, 32). These data suggest that insulin-induced gene expression patterns are associated with poor patient outcome after antiestrogen therapy.

Because InsR and IGF-IR elicit both overlapping and distinct cellular processes (2), we compared insulin-stimulated gene expression to the IGF-I–stimulated gene expression patterns...
reported by Creighton and colleagues, where MCF-7 cells were treated with IGF-I for 3 or 24 hours (33). Common intrinsic pathways and gene sets (i.e., “metagenes”) are coordinately modulated and tend to show better reproducibility and consistency than individual genes (34, 35). Therefore, we carried out gene set analysis (GSA; ref. 36) on each data set followed by hierarchical clustering of the gene set scores instead of individual genes to identify concordant/discordant transcriptional processes. Similar to findings reported by Loboda and colleagues (37), we observed that insulin and IGF-I altered common gene sets following short-term treatment. In contrast, more distinct patterns were apparent after 24 hours (Fig. 7B; Supplementary Fig. S12). Several gene sets enriched after 24 hours of IGF-I comprised cell-cycle-related pathways. In contrast, 24 hours of insulin enriched for gene sets comprising cell metabolism, glycolysis, and pentose-phosphate pathway shunting. These data imply that IGF-IR and InsR elicit both common and distinct transcriptional outputs.
Figure 7. Insulin/IGF-I-induced gene expression correlates with patient outcome after endocrine therapy. MCF-7 cells were serum-starved for 24 hours, and then treated with or without 10 μg/mL insulin for 4 or 24 hours. RNA was isolated and analyzed by using gene expression microarrays. A, a tumor signature of insulin-induced gene expression correlates inversely with RFS in patients with ER⁺ breast cancer treated with tamoxifen. B, genes altered by insulin or IGF-I stimulation were evaluated by GSA. Gene sets were grouped by hierarchical clustering and shown as a heatmap. C, an insulin/IGF-I gene expression signature predicts RFS in 2 cohorts of patients with ER⁺ breast cancer treated with tamoxifen.
Finally, we examined whether a common signature of genes regulated by both ligands was predictive of patient outcome. Similar processing of the published IGF-I data of Creighton and colleagues (33) identified a common set of 155 genes altered by both ligands after short- or long-term treatment. The insulin/IGF-I gene signature correlated inversely with RFS in both cohorts of tamoxifen-treated patients (MDACC298 $P = 0.0004$; LOH164 $P = 0.033$; Fig. 7C). Notably, the insulin/IGF-I gene signature was more predictive of RFS than the insulin signature in both data sets, consistent with the notion that hyperactivation of both receptors generates resistance to endocrine therapy and further implying that both InsR and IGF-IR should be inhibited for reversal or attenuation of such resistance.

**Discussion**

Using a kinome-wide siRNA screen, we identified the InsR/IGF-IR pathway as a mechanism of escape from hormone dependence in ER$^+$ breast cancer. RNAi-mediated knockdown of InsR and/or IGF-IR inhibited growth of ER$^+$ breast cancer cells adapted to hormone deprivation, but dual knockdown additively suppressed PI3K/AKT signaling. Pharmacologic blockade of InsR/IGF-IR with OSI-906 inhibited PI3K/AKT and LTED cell growth. OSI-906 also prevented the emergence of hormone-independent tumors, and suppressed growth of ER$^+$ xenografts in ovariectomized mice. Blockade of IGF-IR alone was insufficient to prevent emergence of hormone-independent cells or suppress tumor growth, suggesting that dual inhibition of InsR and IGF-IR is necessary to prevent escape of ER$^+$ breast cancer cells from estrogen dependence. Combined inhibition of ER and InsR/IGF-IR suppressed hormone-independent tumor growth more effectively than each intervention alone. Finally, an insulin/IGF-I–induced gene expression signature was predictive of RFS in patients with ER$^+$ breast cancer treated with adjuvant tamoxifen.

Although the IGF-IR has been implicated in tamoxifen resistance (7, 29, 38, 39), we show herein the importance of InsR in acquired resistance to endocrine therapy, as a dual inhibitor of InsR/IGF-IR was clearly superior at abrogating hormone dependence compared with a neutralizing IGF-IR antibody. There is evidence of hyperactivation of the InsR/IGF-IR pathway in LTED cells (13), which is likely to be causally associated with resistance to estrogen deprivation. Both InsR and IGF-IR knockdown inhibited hormone-independent growth (Fig. 2A), suggesting that both receptors are important in endocrine-resistant cells. Of note, IGF-IR was not a hit in the siRNA screen; however, false negatives are unavoidable in screens of this nature. IGF-IR knockdown using an independent siRNA suppressed hormone-independent growth (Fig. 2A). Although dual knockdown additively suppressed PI3K/AKT, InsR knockdown inhibited MCF-7/LTED growth more effectively than dual InsR/IGF-IR knockdown, but this difference did not reach statistical significance (Fig. 2A and B). We speculate that the increased effect of InsR knockdown may be due to downregulation of both InsR homodimers and InsR/IGF-IR heterodimers.

The InsR/IGF-IR TKI OSI-906 is in early clinical trials, where it has been well tolerated (40–42). Consistent with observations of hyperglycemia in patients treated with other IGF-IR inhibitors (8, 23), hyperglycemia was reported in a fraction of patients treated with OSI-906 in phase I trials (41, 42). However, this side effect did not limit establishment of a maximum tolerated dose, based on dosing schedules corresponding to drug exposures predicted to inhibit IGF-IR and InsR in tissue and peripheral blood. Treatment with OSI-906 was superior to the IGF-IR antibody MAB391 at inhibiting P38/AKT (Fig. 5A and B). Furthermore, OSI-906 prevented the emergence of hormone-independent cells and tumors (Figs. 4A and 5C), and suppressed hormone-independent tumor growth (Fig. 6A). These results imply that blockade of IGF-IR alone is insufficient to prevent or treat endocrine-resistant breast cancer, and that both receptors should be targeted in this clinical setting. In agreement with these data, a recent report showed that OSI-906 was superior to MAB391 against human colon cancer xenografts (6). Additionally, dual inhibition of InsR/IGF-IR was required to inhibit growth in IGF-II–driven cancers in a transgenic mouse model (43).

The requirement of targeting both InsR and IGF-IR to suppress estrogen-independent tumor growth may help explain the outcome of a recent clinical trial. Patients with ER$^+$ metastatic breast cancer who progressed on prior endocrine therapy were randomized to the AI letrozole with or without the IGF-IR monoclonal antibody AMG-479. AMG-479 did not add to the clinical effect of letrozole alone (44). Although insulin levels were not reported in the AMG-479 study, we speculate that a compensatory upregulation of insulin (Fig. 6C; ref. 23) and, in turn, InsR activation may have negated a clinical effect of the antibody.

Other studies have shown that amplified InsR signaling conveys intrinsic resistance to IGF-IR inhibitors (43, 45). InsR and IGF-IR cross-talk bidirectionally, suggesting that InsR can compensate for loss of IGF-IR (Fig. 2B). Furthermore, IGF-IR downregulation sensitizes breast cancer cells to insulin action (46), MAB391 treatment results in a compensatory increase in InsR phosphorylation (6), and IGF-IR knockout can sensitize cells to insulin-mediated activation of InsR, AKT, and mitogen-activated protein kinase (47). These data further suggest a dual InsR/IGF-IR inhibitor such as OSI-906 would be a better strategy at inhibiting this receptor network. The relative contribution of InsR and IGF-IR homo- versus heterodimers to breast cancer cell growth is unclear. IGF-I and IGF-II bind heterodimers and IGF-IR homodimers with high affinity, whereas insulin binds InsR homodimers but not IGF-IR homodimers or heterodimers at physiologic concentrations (5). Because OSI-906 blocked insulin- and IGF-I–induced P38/AKT activation and cell growth (Fig. 5A and D), we speculate that OSI-906 likely inhibits both InsR and IGF-IR heterodimers and homodimers. Furthermore, insulin and IGF-I altered both common and distinct gene expression signatures, reinforcing distinct functionality of these 2 pathways (Fig. 7B). We speculate that genes commonly deregulated by short-term insulin and IGF-I stimulation may drive resistance to endocrine therapy, because the insulin/IGF-I gene signature was...
more predictive than the insulin signature of disease recurrence (Fig. 7A and C). Collectively, these data suggest that homo- and heterodimers may promote endocrine resistance, and targeting both receptors is required for effective suppression of the InsR/IGF-IR pathway.

In summary, we have identified the InsR/IGF-IR pathway as a mechanism of escape from hormone dependence in ER+ breast cancer. Because inhibition of InsR and IGF-IR prevented the emergence of hormone-independent tumors, we propose early intervention with combined ER and InsR/IGF-IR–directed therapies in high-risk patients with ER+ breast cancer may prevent disease recurrence. Furthermore, this study suggests that targeting InsR/IGF-IR may be more effective than targeting IGF-IR alone. As a result, dual TKIs of InsR/IGF-IR should be more effective than neutralizing IGF-IR antibodies in preventing escape of ER- breast cancer from hormone dependence.

Disclosure of Potential Conflict of Interest

H.C. Manning received a commercial grant from OSI Pharmaceuticals. The other authors disclosed no potential conflicts of interest.

Grant Support

This work was supported by American Cancer Society Postdoctoral Fellowship grant PF-10-184-01-TBE (E.M. Fox), NH $32CA129100 (T.W. Miller), K99CA143999 (T.W. Miller), Breast Cancer Specialized Program of Research Excellence (SPOR) grant P50CA096131, Vanderbilt-Ingram Cancer Center Support grant P30CA64845, U24CA126588 (South-Eastern Center for Small-Animal Imaging), RO1CA146028 (H.C. Manning), RC1CA145138 (H.C. Manning), K25CA127349 (H.C. Manning); a Breast Cancer Research Foundation grant (C.L. Arteaga); ACS Clinical Research Professorship grant CBF-07-234 (C.L. Arteaga); the Lee Jeans Translational Breast Cancer Research Program (C.L. Arteaga); Stand Up To Cancer/AACR Dream Team Translational Cancer Research Grant SU2C-AACR-DT0209 (C.L. Arteaga. G.B. Mills, A.M. Gonzalez-Angulo).

Received April 18, 2011; revised August 19, 2011; accepted September 6, 2011; published OnlineFirst September 9, 2011.

References

A Kinome-Wide Screen Identifies the Insulin/IGF-I Receptor Pathway as a Mechanism of Escape from Hormone Dependence in Breast Cancer

Emily M. Fox, Todd W. Miller, Justin M. Balko, et al.

Cancer Res  Published OnlineFirst September 9, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-11-1295

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2011/09/09/0008-5472.CAN-11-1295.DC1

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.