Src Inhibition Blocks c-Myc Translation and Glucose Metabolism to Prevent the Development of Breast Cancer

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Abstract

Preventing breast cancer will require the development of targeted strategies that can effectively block disease progression. Tamoxifen and aromatase inhibitors are effective in addressing estrogen receptor–positive (ER+) breast cancer development, but estrogen receptor–negative (ER–) breast cancer remains an unmet challenge due to gaps in pathobiologic understanding. In this study, we used reverse-phase protein array to identify activation of Src kinase as an early signaling alteration in premalignant breast lesions of women who did not respond to tamoxifen, a widely used ER antagonist for hormonal therapy of breast cancer. Src kinase blockade with the small-molecule inhibitor saracatinib prevented the disorganized three-dimensional growth of ER– mammary epithelial cells in vitro and delayed the development of premalignant lesions and tumors in vivo in mouse models developing HER2+ and ER– mammary tumors, extending tumor-free and overall survival. Mechanistic investigations revealed that Src blockade reduced glucose metabolism as a result of an inhibition in ERK1/2– MNK1–eIF4E–mediated cap-dependent translation of c-Myc and transcription of the glucose transporter GLUT1, thereby limiting energy available for cell growth. Taken together, our results provide a sound rationale to target Src pathways in premalignant breast lesions to limit the development of breast cancers. Cancer Res; 75(22); 4863–75. ©2015 AACR.

Introduction

Despite recent advances in effective treatment, including targeted therapies, many women still die of breast cancer (1). Ultimately, the most effective way to reduce breast cancer mortality is disease prevention (2). In large-scale prevention trials, tamoxifen and aromatase inhibitors reduced the incidence of estrogen receptor–positive (ER+) breast cancer in high-risk women by approximately 30% (3, 4). However, prevention of estrogen receptor–negative (ER–) and tamoxifen-resistant (TamR) breast cancer remains an overarching unmet demand. ER– breast cancers account for approximately 30% of total breast cancers (5), of which EGFR2-positive (HER2+)/ER– and triple-negative breast cancer subtypes account for approximately 10% to 15% and 15% to 20% of total breast cancers, respectively (5, 6). Here, we explored strategies to prevent ER– breast cancer in an HER2+/ER– mammary tumor model.

One of the molecular mechanisms of ER loss in ER– breast cancer is constitutive proteolysis of ERα via Src activation (7). Moreover, Src promotes ER phosphorylation, leading to increased proliferation and tamoxifen resistance in breast cancer cells (8, 9). ER– breast cancer cells have been found to be more dependent on Src activation than ER+ breast cancer cells and noncancerous breast cells, as Src silencing increased ER– breast cancer cell death (10). In TamR breast cancer cells, Src activity is upregulated and associated with a more aggressive phenotype (11). These findings indicate that Src activation is a key signaling event driving ER– breast cancer. Src targeting agents, such as the small-molecule tyrosine kinase inhibitors dasatinib, bosutinib, and saracatinib, have been extensively tested in the clinic for treatment of metastatic breast cancer (12); however, they have never been explored for cancer prevention purposes. Given the benefits associated with targeted cancer therapies, for example, better tolerance and lower cost of small-molecule inhibitors relative to chemotherapy (13), we postulated that low-dose Src targeting agents may be an effective and well-tolerated option for prevention of ER– breast cancer. Dasatinib and bosutinib are active against a broad spectrum of kinases, whereas saracatinib is a potent and more selective Src inhibitor (13), making it a good candidate for proof-of-concept prevention studies. In addition, saracatinib used in combination with fulvestrant (ER antagonist), circumvented antiestrogen resistance in ER– breast and ovarian cancer preclinical models (14, 15). Given...
the lack of low-toxicity targeted agents for ER− breast cancer prevention (16), saracatinib at lower doses may possess unrecognized potential for breast cancer prevention.

Metabolic dysregulations have been associated with increased breast cancer risk (17). Premalignant and neoplastic cells exhibit increased demand for energy and nutrients to proliferate and survive (18, 19). Increased glucose uptake enables the generation of building blocks for dysregulated cellular growth, which facilitates cancer initiation (18). In tumor cells, c-Myc (Myc) plays an important role in regulating glycolysis and glutaminolysis (20). Endocrine-resistant breast cancer cells overexpress Myc, which correlates with increased dependency on glucose and glutamine, and these cells could survive on glutamine upon glucose deprivation (21). Notably, ER− primary breast tumors had an increased uptake of 18F-fluorodeoxyglucose (a glucose analogue) and express higher levels of glucose transporter 1 (GLUT1) than ER+ tumors (22, 23). However, the role of glucose metabolic dysregulation in the early stages of cancer is unclear, and the role of Src in regulating glucose metabolism in cancer cells is not well studied.

In this study, we set out to tackle the challenges of preventing HER2+ and ER− breast cancer. We found that Src activation was a key signaling alteration during early-stage cancer initiation and that Src inhibition suppressed cap-dependent translation of Myc and reduced GLUT1 transcription and glucose uptake in premalignant ER− mammary epithelial cells (MEC), consequently inhibiting cell proliferation and ER− mammary tumor initiation and development. These preclinical findings provide a strong scientific foundation of using Src inhibitors for preventing, at least this subtype, of ER− breast cancer.

Materials and Methods
Patient samples

Patient sample collection was carried out in accordance with institutional review board-approved protocol at the Duke University Medical Center (Durham, NC). "High-risk patients" include women with greater than 20% lifetime risk of developing breast cancer, as assessed by at least one of the criteria: (i) prior biopsy containing atypia, ductal carcinoma in situ (DCIS), or lobular carcinoma in situ (LCIS), (ii) known or suspected to have BRCA mutations, or (iii) first-degree family member with premenopausal breast cancer. No BRCA mutation carriers were included in this study. The presence of atypia in random perineural and ductal carcinoma in situ (PNI) has been used as a surrogate marker to track cytologic response to chemoprevention agents. Informed consent was obtained before enrolling women in the study. In the following analysis, ER status was not determined in any of the materials collected from patients.

Cells and vectors

The ER− mammary epithelial cells, MCF10A and MCF12A, were obtained from ATCC. The pLKO.1-based shRNAs for Src were purchased from Sigma-Aldrich and the Src-mutant (Y527F) construct from Addgene. shRNAs for GLUT1 were obtained from MD Anderson Cancer Center's shRNA core facility (Houston, TX).

Amplex Red glucose assay kit

The Amplex Red Glucose Assay Kit was used (Life Technologies) following previously published protocol (24). Briefly, 1.3 × 105 cells were plated in poly-HEMA–coated 96 wells. After 24 hours, media were collected and diluted 1:4,000 in water. The amount of glucose in the media was then determined using the Amplex Red Assay according to the manufacturer's instructions. Glucose uptake was analyzed by subtracting the amount of glucose in each sample from the total amount of glucose in the media (without cells). The data represent experiments from three independent replicates. To examine the effect of saracatinib on glucose uptake, cells pretreated with either vehicle or saracatinib for 3 days were seeded to perform the assay.

ER− mammary tumor prevention studies

Mouse experiments were performed in accordance with approved protocols from the Institutional Animal Care and Use Committee of MD Anderson Cancer Center. Female MMTV-neu mice were treated with either vehicle (0.5% hydroxypropyl methylcellulose with Tween-80) or saracatinib by oral gavage once daily for 6 days a week. Tumor sizes were measured twice a week. Tumor-free survival was defined as the time from date of birth to the first appearance of a palpable mammary tumor at least 100 mm3 in size. The fourth pair of normal looking mammary fat pads (MFP) was isolated from these mice at 32 weeks of age. For histologic analyses, nonserial sections thoughtout the MFPs were analyzed. Another cohort was set up using female MMTV-neu− mice. These mice were treated either with vehicle or saracatinib. Tumor-free and overall survivals were monitored.

Statistical analyses

Quantitative results were analyzed either by one-way ANOVA (multiple groups) or t test (two groups). Differences with \( P < 0.05 \) (two-sided) were considered statistically significant. \(*, P < 0.05; **, P < 0.01; *** P < 0.001. \) For patient samples, Wilcoxon rank-sum test was used. Tumor-free and overall survival analyses were performed using the Kaplan–Meier Wilcoxon test. Bars represent means ± SEM.

Results

Elevated Src expression in premalignant breast lesions of women who did not respond to tamoxifen

To develop effective prevention strategy for ER antagonist–unresponsive breast cancer, we sought to identify targetable molecular signature in premalignant lesions of women who did not respond to tamoxifen and were at a higher risk of developing tamoxifen-unresponsive breast cancer. Eighteen high-risk women...
Targeting Src prevents disorganized growth of ER+ MECs

Because HER2-overexpressing MECs exhibit higher Src activation (27), to determine the role of Src in ER+ preneoplastic MECs growth, we generated ER+ MCF10A and MCF12A MECs stable clones harboring HER2 overexpression (10A.B2 and 12A.B2) or control vectors (10A.vec and 12A.vec) as in vitro models (Supplementary Fig. S1D; ref. 27). In three-dimensional (3D) culture, 10A.B2 and 12A.B2 cells form noninvasive disorganized acinar structures with filled lumen due to increased proliferation and reduced apoptosis compared with the control cells (Fig. 1B, second column from left and Supplementary Fig. S1G, middle column). These acinar structures mimic DCIS in patients (27) and can be used for testing therapeutics (28). On the contrary, the vector control cells form spherical acinar structures with a hollow lumen that mimic normal mammary glands in vivo (Fig. 1B and Supplementary Fig. S1G, left columns; ref. 29). To determine the role of Src in the disorganized acinar growth of 10A.B2 cells, we knocked down Src in 10A.B2 cells (Supplementary Fig. S1E). In 3D culture, 10A.B2 cells and control shRNA (ctrl.shRNA)-expressing 10A.B2 cells formed disorganized acinar structures, whereas Src knockdown (Src.sh) resulted in smaller spherical acini structurally similar to those of the 10A.vec cells (Fig. 1B and C). Staining of markers for apoptosis (cleaved caspase-3), proliferation (Ki-67), and basement membrane (laminin 5) showed that acini formed by 10A.B2.Src.sh cells exhibited fewer proliferating cells and more apoptotic cells than those of 10A.B2.ctrl.shRNA cells (Fig. 1B and C). These data demonstrated that Src is required for apoptosis resistance, MEC proliferation, and the disorganized acinar growth of 10A.B2 cells in 3D culture. Next, we tested the Src inhibitor saracatinib for prevention of the growth of disorganized acini in Src-activated MECs (Fig. 1D). We first confirmed that saracatinib indeed inhibited activation of Src and its downstream targets, such as phospho-FAK-Y576, phospho-P130 Cas-Y410, and phospho-Paxillin-Y118 in both 10A.B2 and 12A.B2 cells in 3D cultures (Supplementary Fig. S1F). We then treated 10A.B2 and 12A.B2 cells with either vehicle or saracatinib (1 μmol/L) on day 6 of 3D culture, when there was no significant difference in acinar growth of 10A.B2 and 12A.B2 cells compared with their vector control cells (Fig. 1D). Saracatinib indeed prevented the disorganized acinar growth of 10A.B2 and 12A.B2 cells, resulting in smaller spherical acinar structures with hollow lumina similar to those of vector control cells (Fig. 1E and F and Supplementary Fig. S1G and S1H). Saracatinib also induced apoptosis and inhibited proliferation of acini, as shown by increased cleaved caspase-3 and decreased Ki-67 and MCM2 (Fig. 1E and Supplementary Fig. S1G and S1I).

Targeting Src delays ER+ mammary tumor development in mice

We next examined the effect of saracatinib on preventing ER+ mammary tumors in the MMTV-neu 202 Mul/J (denoted as MMTV-neu, neu is the rat homologue of human Erbb2/HER2) mouse model (30). MMTV-neu mouse overexpresses wild-type HER2 in the mammary gland and progressively develops ER+ mammary intraepithelial neoplasia (MIN; ~10–18 weeks) and invasive ductal carcinomas (IDC, ~30 weeks onward) lesions (data not shown). As the well-tolerated saracatinib dose used for cancer treatment in clinical trials is 175 mg/d (equivalent to 33.2 mg/kg in mouse; ref. 31), we used saracatinib at a lower dose (25 mg/kg, n = 20) for treating MMTV-neu mice and gave vehicle to control group (n = 20) and monitored the mice for tumor-free survival. All mice in the vehicle group developed mammary tumors by 260 days of age, when 9 of 20 mice in the saracatinib-treated group remained tumor-free (Fig. 2A). Compared with the vehicle group, saracatinib significantly increased tumor-free survival [vehicle; T(50) = 211 days, saracatinib; T(50) = 249 days] and overall [vehicle; T(50) = 258 days, saracatinib; T(50) = 285 days] survival of these mice (Fig. 2A and Supplementary Fig. S2A) without obvious toxicity to mice (ref. 32 and data not shown). Saracatinib significantly reduced the development of hyperplastic, MIN, and IDC lesions (Fig. 2B). Immunohistochemical (IHC) staining confirmed that saracatinib treatment inhibited Src activity in MFPs (Supplementary Fig. S2C).

To monitor mammary tumor progression from hyperplasia to MIN to IDC during saracatinib treatment in real-time, we implemented the high-resolution micro-endoscopy (HRME), an innovative imaging technology, which has been used as a diagnostic tool to distinguish normal tissue from benign and neoplastic tissues in head and neck and cervical cancers (33, 34). Fresh MFPs from 32-week-old vehicle- and saracatinib-treated mice were imaged to detect premalignant lesions using HRME with or without structured illumination (SI), a method of rejecting out-of-focus light to improve image contrast in thick tissue samples. The images of HRME with SI (HRME-SI) were co-registered with images obtained from confocal microscopy and hematoyxlin and eosin (H&E) staining for comparison of resolutions (Supplementary Fig. S2B). The quality of images from HRME-SI, which could be applied in real-time in living animals through a biopsy needle, was comparable with that of confocal microscopy and H&E staining. The HRME-SI detected MIN lesions in the vehicle-treated MMTV-neu mice, whereas only hyperplastic lesions were detected in the saracatinib-treated mice (Supplementary Fig. S2B).
We also tested the effect of saracatinib in MMTV-neu NDL 2–5 (referred to as MMTV-neu) mouse model expressing the activated neu as female MMTV-neu mice rapidly develop ER− mammary tumors (35). Specifically, MMTV-neu female mice develop hyperplastic lesions between 8 and 11 weeks, MIN lesions between 11 and 19 weeks, and IDC lesions between 19 and 26 weeks of age (Fig. 2D). IHC staining of MFP biopsies showed that MMTV-neu mice developed atypia and MIN lesions with a gradual loss of ER and increase in Src activation as disease progressed to mammary tumors (Fig. 2D).

Given the appearance of MIN lesions between 11 and 19 weeks and an enhanced Src activation (Fig. 2D) in MIN lesions of MMTV-neu mice, we tested saracatinib for prevention of the development of ER− IDC in this model by starting treatment in 10-week-old female mice. We gave saracatinib (25 mg/kg, n = 20) to MMTV-neu mice and used vehicle in control group (n = 20). Compared with vehicle treatment, saracatinib significantly increased the median tumor-free [vehicle; T(50) = 155 days, saracatinib; T(50) = 176 days] and overall [vehicle; T(50) = 176 days, saracatinib; T(50) = 196 days] survival of mice (Supplementary Fig. S2C and S2D). To determine whether saracatinib inhibited Src activation and the appearance of hyperplastic, MIN, and IDC lesions, the MFPs were collected at 21 weeks of age from vehicle- and saracatinib-treated mice when there was abundance...
Figure 3.
of hyperplastic and MIN lesions but less than 10% IDC lesions. Indeed, low-dose saracatinib treatment reduced Src activation (Supplementary Fig. S2E). MFPS from saracatinib-treated mice exhibited significantly reduced number of IDC lesions and fewer MIN lesions than vehicle-treated mice (Supplementary Fig. S2F). Altogether, these data clearly demonstrated that saracatinib delayed initiation and progression of premalignant lesions and increased both tumor-free and overall survival in two mouse models of ER- and Src-activated mammary tumors.

**Src regulates glucose metabolism and GLUT1 expression**

Because metabolic alterations are prominent in early-stage cancers (17), to gain mechanistic insights into the delayed initiation and progression of premalignant lesions by targeting Src, we performed metabolomic profiling of MFPS of vehicle- and saracatinib-treated MMTV-neu® mice. Metabolite set enrichment analysis showed that starch and sucrose metabolism, the pentose phosphate pathway, and glycolysis were among the most significantly downregulated pathways in response to Src inhibition (Supplementary Fig. S3A). Glucose 6-phosphate and fructose 6-phosphate were among the most inhibited metabolites in response to saracatinib treatment (Supplementary Fig. S3B and S3C). Given that glucose 6-phosphate is the common metabolite of these pathways, we hypothesized that Src inhibition may affect the availability of glucose to premalignant cells, which would in turn affect the synthesis of downstream metabolites of the aforementioned pathways. Therefore, we compared glucose uptake by 10A.B2 and 12A.B2 cells to their corresponding control cells with or without saracatinib treatment. Compared with vector control cells, 10A.B2 and 12A.B2 MECs had a significant increase in glucose uptake, which was inhibited by saracatinib treatment (Fig. 3A and B). Furthermore, knocking down Src (Supplementary Fig. S1E and S3D) also significantly reduced the glucose uptake compared with control shRNA transfected cells (Fig. 3C and D). These data indicate that Src plays an essential role in regulating glucose uptake.

Transport of glucose across the plasma membrane of mammalian cells is carried out by glucose transporter (GLUT) proteins that are widely dysregulated in many cancers (36). Mammary glands predominantly express GLUT1, which also facilitates transport of mannose and galactose in addition to glucose (37). Particularly, GLUT1 is expressed at much higher levels compared with other...
High GLUT1 expression is required for disorganized acinar growth of ER- and Src-activated MECs

To determine whether GLUT1 is critical for glucose uptake in ER- and Src-activated MECs, we stably knocked down GLUT1 in 10A.B2 cells (10A.B2.GLU1.sh, Fig. 4A). Clearly, 10A.B2.GLU1.sh cells had reduced glucose uptake compared with control cells (Fig. 4B), indicating that GLUT1 is the major glucose transporter responsible for glucose uptake in 10A.B2 cells. Remarkably, 10A.B2.GLU1.sh stable cells did not form disorganized acinar structures as 10A.B2 and control shRNA transfected 10A.B2 cells did in 3D culture (Fig. 4C, top, and 4D). Moreover, immunofluorescent staining showed that silencing GLUT1 resulted in increased apoptosis and decreased proliferation in acini of 10A.B2 cells (Fig. 4C, bottom). These data indicated that GLUT1 plays an important role in disorganized acinar growth of ER- Src-activated 10A.B2 cells by increasing glucose uptake to provide energy.

Ssrc upregulates GLUT1 expression through increasing c-Myc protein synthesis

To gain mechanistic insights on how Src inhibition reduced GLUT1 expression and glucose uptake (Fig. 3), we treated 10A.B2 cells grown in 3D culture with either vehicle or saracatinib and performed RPPA. Among many detected alterations, c-Myc was one of the top three transcriptional regulators that were downregulated by saracatinib treatment (Supplementary Table S1). Myc downregulation by saracatinib was validated by immunoblotting in both 10A.B2 and 12A.B2 cells (Fig. 5A). As Myc had been reported to transcriptionally upregulate GLUT1 expression in Rat-1 fibroblasts (40), we examined whether Myc regulated GLUT1 expression in 10A.B2 and 12A.B2 cells. Myc silencing by siRNA resulted in decreased GLUT1 at both the protein and mRNA levels in 10A.B2 and 12A.B2 cells (Fig. 5B–D). As the data clearly demonstrated that Myc regulates GLUT1 expression in these MECs, we further investigated how Src inhibition affected Myc expression. Although saracatinib inhibited Myc protein levels of 10A.B2 and 12A.B2 cells, it did not inhibit Myc mRNA expression (Fig. 5A and Supplementary Fig. S4A). Myc protein expression was also dramatically reduced in 3D-cultured 10A.B2 Src.sh and 12A.B2 Src.sh cells compared with control shRNA transfected cells (Fig. 5E). In addition, Src.wt- and Src.Y527F transfected MCF10A and MCF12A cells showed increased Myc protein expression compared with vector control cells but no significant changes in Myc mRNA levels (Fig. 5F and Supplementary Fig. S4B). These data indicated that Src regulates Myc at the protein level. Furthermore, Myc protein expression significantly correlated with phospho-Src-Y416 level, among human breast tumors in the TCPA dataset (Supplementary Fig. S4C).

The combination of protein synthesis and stability determines steady-state protein levels (41). To examine whether Src inhibition affects Myc protein stability, vehicle- or saracatinib-treated 12A.B2 MECs were treated with cycloheximide to block protein synthesis and cell lysates were collected at different treatment times (0–60 minutes) followed by Myc immunoblotting. Similar rates of Myc protein degradation were detected between vehicle- and saracatinib-treated cells (Supplementary Fig. S4D), indicating that Src inhibition did not reduce the stability of Myc protein.

To determine the effect of Src inhibition on Myc protein synthesis, polysome fractions were collected from vehicle- and saracatinib-treated 10A.B2 and 12A.B2 cells. Saracatinib did not significantly change the overall polysome profile compared with vehicle controls (comparison of Fig. 5G with 5H and Supplementary Fig. S4E with S4F), indicating that Src inhibition did not affect global protein synthesis. However, saracatinib drastically attenuated polysomal recruitment of Myc mRNA compared with vehicle (Fig. 5I and J and Supplementary Fig. S4G and S4H), indicating that saracatinib inhibited Myc protein translation in these MECs.

Ssrc increases Myc protein by enhancing cap-dependent translation

The synthesis of many oncopgenic proteins is promoted by cap-dependent translation, which is regulated by phosphorylated elf4E (42–44). The elf4E is phosphorylated at serine 209 by MAPK signal integrating kinases 1 and 2 (MNK1 and MNK2;
MNK2 is constitutively active, whereas MNK1 is activated by external stimuli via ERK and p38 mitogen-activated protein kinase (MAPK; ref. 45). To investigate whether saracatinib inhibited Myc translation by inhibiting cap-dependent translation, we compared phospho-eIF4E-S209 and its upstream kinases in vehicle- or saracatinib-treated 10A.B2 and 12A.B2 cells. Saracatinib suppressed phospho-eIF4E-S209 and its upstream kinases phospho-MNK1-T197/202 and phospho-ERK1/2-T202/Y204, but not phospho-p38 MAPK-T180/182, suggesting that Src affects cap-dependent translation via the ERK1/2–MNK1–eIF4E pathway (Fig. 5K). Furthermore, eIF4E-silenced 10A.B2 and 12A.B2 cells showed decreased levels of Myc protein, confirming cap-dependent translation of Myc in these cells (Fig. 5L). Thus, saracatinib reduced cap-dependent translation of Myc by inhibiting the ERK1/2–MNK1–eIF4E pathway.

Saracatinib inhibits Myc and GLUT1 expression in mouse models

Next, we set out to determine whether the above findings from 3D cultured MECs recapitulated the effects of targeting Src with saracatinib in mouse models. MFPs collected from vehicle- and saracatinib-treated MMTV-neu and MMTV-neuβ mice were compared for Ki-67, Myc, and GLUT1 expression by IHC staining. Compared with vehicle-treated mice, Ki-67 in MFPs from saracatinib-treated mice was significantly reduced (Fig. 6A and Supplementary Fig. S5A), consistent with the delayed tumor onset and progression observed in these mice (Fig. 2A and Supplementary Fig. S2A, S2C, and S2D). Myc was significantly reduced in MFPs of saracatinib-treated mice (Fig. 6B and Supplementary Fig. S5B) with a corresponding reduction of GLUT1 expression (Fig. 6C and Supplementary Fig. S5C). Together, these in vivo findings were consistent with the Src inhibition–mediated responses in 3D cultured 10A.B2 and 12A.B2 cells, indicating that targeting Src with saracatinib inhibited Myc expression, resulting in reduced GLUT1 expression, consequently impeding glucose metabolism and ERβ Src-activated mammary tumor initiation and progression.

Discussion

Despite recent advances in treatment, breast cancer accounts for an estimated 29% (232,670) of new cancer cases, highest among all female-related cancers, and 15% (40,000) of cancer deaths per year (1). Effective prevention strategies are needed to reduce breast cancer deaths. The development of agents to prevent tamoxifen-unresponsive and ERβ breast cancer requires better understanding of the critical molecular alterations driving early lesion (atypia) progression toward breast cancer. In this study, we found that Src activation is a targetable molecular alteration in early-stage Tam-NR atypical lesions of women at higher risk of developing tamoxifen-unresponsive breast cancer. Here, we show that saracatinib effectively targets Src and prevents the disorganized acinar growth of HER2-overexpressing, Src-activated, and ERβ MECs in 3D culture, a condition mimicking the growth of premalignant lesions in early-stage breast disease. Importantly, saracatinib markedly prevented the development of premalignant lesions and delayed tumor onset in two ERβ mammary tumor mouse models with HER2-induced Src activation. These strong preclinical data have led to the recent initiation of a clinical trial using a Src inhibitor for secondary prevention of ERβ breast cancer at MD Anderson Cancer Center (ClinicalTrials.gov identifier: NCT01471106).

Previous RPPA analysis in primary breast tumors showed significantly higher Src expression in ERβ– and progesterone receptor–negative (PR–) tumors than in ERβ+ and/or PR+ tumors, and ERβ and Src expression were inversely correlated in all tumors (46). Deletion of Src in epithelial cells delayed polyomavirus middle-T antigen (PyVmT)-driven ERβ mammary tumorigenesis (47). Recently, dasatinib was shown to delay mammary tumor onset driven by activated HER2 and PTEN loss.
tumor models support our findings (48). These studies in advanced breast cancer and mammary tumor models support our findings that Src plays an important role in ER− breast cancer progression. However, our current study represents the first discovery that Src activation is a readily targetable event in women who are at high risk of developing tamoxifen-unresponsive breast cancer and that Src inhibition effectively prevents the progression of ER− early breast lesions.

Cancer metabolism is a recently recognized hallmark of cancer, as chronic and uncontrolled cell proliferation often also involves dysregulation of energy metabolism (49). Recently, clinical trials implementing caloric restriction and exercise have shown promising results for the prevention of breast cancer in high-risk women (50). Because of the behavioral difficulty in maintaining a CR diet for a long period of time, currently there is a focus on identifying and developing agents that could complement, synergize, or mimic the anticancer effects of caloric restriction (51). Our metabolomic data showed for the first time that in an ER− breast cancer model, Src regulates carbohydrate metabolism, partly by regulating glucose uptake. Our data revealed that Src activation increases glucose uptake via upregulating GLUT1 and that saracatinib inhibits glucose uptake by downregulating GLUT1, indicating that targeting Src could help to achieve the goals of caloric restriction by reducing glucose uptake.

Previous microarray analysis showed that genes associated with increased glucose metabolism significantly overlap with those of an ER− molecular phenotype (22), suggesting that increased glucose metabolism contributes to ER− breast cancer development. In this study, we were surprised to find that GLUT1 silencing alone prevented the abnormal acinar growth of ER− 10A.B2 cells, indicating that GLUT1 is required for disorganized acinar growth. Our data provided direct evidence of the critical role of glucose metabolism in dysregulated growth of ER− MECs. Mechanistically, our data showed that Src-mediated GLUT1 upregulation is Myc-dependent. We revealed that Src promotes cap-dependent translation of Myc protein via activation of the ERK1/2–MNK1–eIF4E pathway (Fig. 7). Although Src was found to increase cap-dependent translation of β-catenin and hypoxia-inducible factor 1α (42, 43), our data demonstrated for the first time that Src enhances the translation of Myc, a strong oncogene activated by various mitogenic signals. Consistent with our in-depth mechanistic findings in vitro, saracatinib also significantly inhibited Src activation and Myc and GLUT1 expression in mouse models.

Together, our data present proof of concept that targeting glucose metabolism can be a promising approach for future prevention strategies of ER− breast cancer.

The detection of premalignant lesions at the early stages of cancer development allows for potential early management. In this study, we applied HRME-SI imaging technology to monitor mammary tumor progression (Supplementary Fig. S2B). Because HRME-SI offers several advantages over traditional measures, for example, low cost, real-time imaging, ease of interpretation at the point-of-care, and visualization of cellular and architectural features (52–54), we have begun testing the HRME-SI imaging technology in human breast cancer surgical specimens, aiming at bringing the pilot imaging strategies to the clinic in the near future.

In summary, we identified Src activation as a targetable alteration for prevention of ER− breast cancer and demonstrated that targeting Src with saracatinib delayed ER− mammary tumor initiation and progression. The prevention strategies developed in this study can be immediately implemented to test a wide range of cancer prevention agents. Our approach here could be ultimately developed to achieve a significant reduction in mortality from ER− breast cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: S. Jain, D. Yu
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