AGTR1 overexpression defines a subset of breast cancer and confers sensitivity to losartan, an AGTR1 antagonist

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Breast cancer patients have benefited from the use of targeted therapies directed at specific molecular alterations. To identify additional opportunities for targeted therapy, we searched for genes with marked overexpression in subsets of tumors across a panel of breast cancer profiling studies comprising 3,200 microarray experiments. In addition to prioritizing ERBB2, we found AGTR1, the angiotensin II receptor type I, to be markedly overexpressed in 10–20% of breast cancer cases across multiple independent patient cohorts. Validation experiments confirmed that AGTR1 is highly overexpressed, in several cases more than 100-fold. AGTR1 overexpression was restricted to estrogen receptor-positive tumors and was mutually exclusive with ERBB2 overexpression across all samples. Ectopic overexpression of AGTR1 in primary mammary epithelial cells, combined with angiotensin II stimulation, led to a highly invasive phenotype that was attenuated by the AGTR1 antagonist losartan. Similarly, losartan reduced tumor growth by 30% in AGTR1-positive breast cancer xenografts. Taken together, these observations indicate that marked AGTR1 overexpression defines a subpopulation of ER-positive, ERBB2-negative breast cancer that may benefit from targeted therapy with AGTR1 antagonists, such as losartan.

A central aim in cancer research is to identify genetic alterations involved in the pathogenesis of cancer, thereby providing an opportunity to develop therapies that directly target the alterations. In breast cancer research, this strategy has been realized with the study of ERBB2, which is amplified and overexpressed in 25–30% of breast tumors (1, 2), directly contributing to tumorigenesis (3, 4). Targeting this genetic lesion with trastuzumab, a humanized monoclonal antibody directed against ERBB2, has significant clinical benefit in breast cancer management (5–7). Cancer genes are activated or inactivated by a variety of mechanisms, including those that alter the activity of proteins (e.g., activating Ras mutation, BCR-ABL fusion protein) and those that change expression levels of proteins (e.g., ERBB2 gene amplification, Ig-Myc DNA translocation, or p53 homozygous deletion). It is likely that only a fraction of such "driver" alterations have been identified to date, and furthermore, many of the identified alterations are not thought to be "druggable" by conventional means.

DNA microarrays have been widely applied to the study of gene expression in cancer. Although microarrays are not capable of directly detecting alterations affecting the activity of proteins, they are theoretically well suited to detect alterations that change the expression of genes and proteins, although it can be difficult to identify driver alterations directly related to tumorigenesis among hundreds or thousands of differentially expressed genes. As a strategy for using microarray data to identify genes directly related to cancer pathogenesis that may thus serve as therapeutic targets, we hypothesized that genes that show the most profound changes in gene expression (10-fold to more than 100-fold increase relative to baseline), termed "pathogenic overexpression," even if in only a small subset of cases, may play a direct role in cancer progression and may serve as optimal therapeutic targets for the subpopulations with overexpression. Because cancer is heterogeneous, distribution statistics that compare average expression values between classes of samples (e.g., cancer vs. normal) will often fail to identify these profound changes in expression, especially if the alterations occur in subsets of cases (e.g., Her2/neu amplification and overexpression in 25% of breast cancer). We previously developed a simple analytical method, termed "Cancer Outlier Profile Analysis" (COPA), to identify such gene expression profiles, nominating ERG and ETV1 as novel cancer genes in prostate cancer, which were shown to be activated by gene fusions with the androgen-regulated gene TMPRSS2 (8). Here, we extend the COPA approach to include a meta-analysis strategy, combining the search for profound changes in expression with multistudy validation. We focus our analysis on breast cancer because this disease has been most extensively analyzed by gene expression profiling. Interestingly, the majority of such analyses have focused on disease classification and prediction of patient outcome, rather than target discovery. We present a large-scale analysis spanning 31 gene expression profiling studies comprising nearly 3,200 microarray experiments. In addition to objectively identifying the prototypical breast cancer target, ERBB2, our analysis also nominates a number of previously unidentified genes which, based on their profound overexpression in subsets of tumors across independent cohorts, may play a role in tumorigenesis and may serve as therapeutic targets in their respective subpopulations.

Results

We hypothesized that genes directly involved in breast tumorigenesis may be activated via pathological overexpression in specific subsets of tumors. Thus, we developed a methodology to

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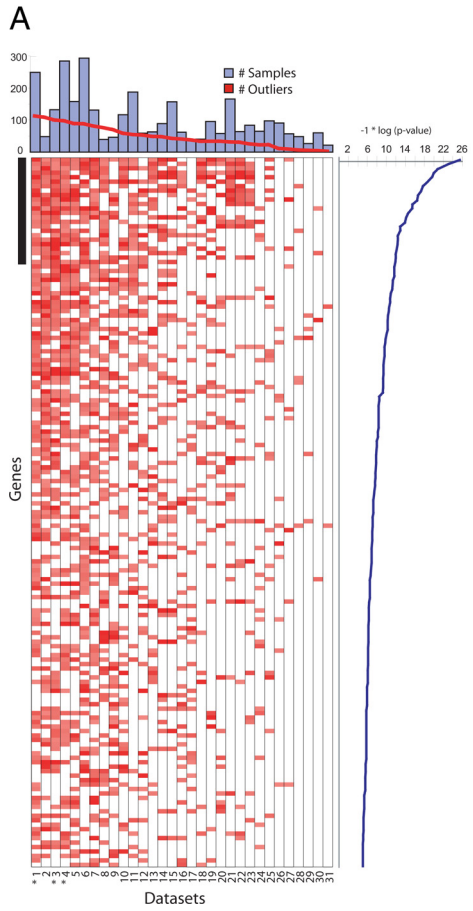

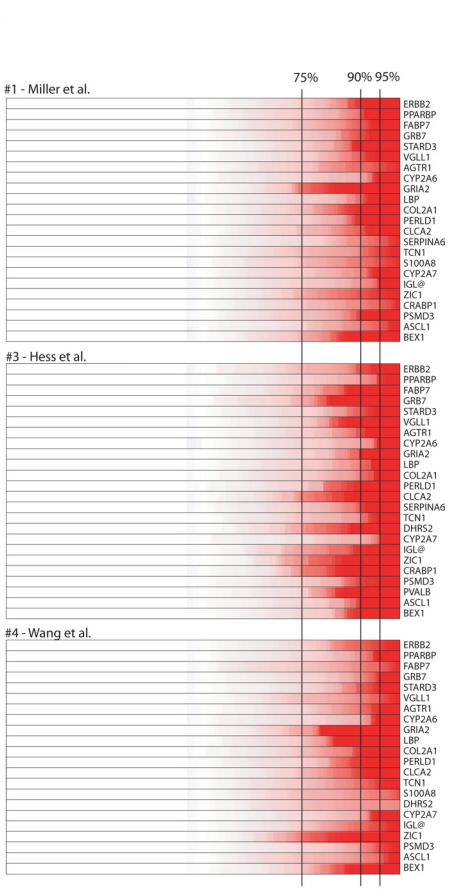

Fig. 1. MetaCOPA analysis of breast cancer gene expression data. (*A*) MetaCOPA map. Each column in the map represents a breast cancer gene expression dataset. The numbers at the base of the map correspond to dataset details (Table S1). Each row indicates a gene. A red cell indicates that the gene was deemed to have an outlier expression profile in the respective dataset because it scored in the top 1% of COPA values at 1 of 3 percentile cutoffs. The line graph along the *y* axis indicates the *P* value for a gene based on the number of datasets in which the gene was deemed an outlier. A total of 158 genes were called outliers in a significant fraction of datasets ($P < 1E\text{-}5$). The bar graph indicates the number of samples in the respective datasets and the contribution of the dataset to the meta-analysis. The black bar on the left of the map indicates the top 25 meta-outliers, which are detailed in *B* for 3 datasets marked with an asterisk. (*B*) Heatmaps of COPA-normalized values for top-scoring meta-outliers across 3 highly contributory datasets: Miller et al. (26), Hess et al. (27), and Wang et al. (28). Genes are ranked by their MetaCOPA *P* values. For each gene, samples are ordered from left to right by their COPA-normalized expression values. Highest intensity of red indicates a COPA-normalized value of 6 or greater. White indicates a value of zero or less.

identify genes that display substantial changes in expression in subpopulations of tumors across independent cancer microarray datasets. The methodology, MetaCOPA, combines MetaAnalysis and COPA, 2 approaches that we have applied previously but separately to identify cancer genes (8, 9) (Fig. S1). We analyzed 31 breast cancer profiling datasets, comprising 3,157 microarrays (Table S1). We defined per dataset "outliers" as genes with the most dramatic overexpression in a subset of tumors, and"meta-outliers" as genes that were identified in a statistically significant fraction of datasets. We identified 159 significant meta-outliers ($P < 1E\text{-}5$) (Fig. 1*A* and Table S2), of which ≈20 genes were identified as outliers in the majority of datasets examined (Fig. 1*B* and Table S3).

Notably, considering all human genes represented in the analysis, ERBB2 was the most significant meta-outlier, identified in 21 of 29 independent datasets (72%; $P = 3.6E\text{-}26$), indicating that this established therapeutic target shows the most substantial and consistent overexpression in a fraction of breast tumors (Fig. S2*A*). Although ERBB2 did not have a no.1 ranked outlier expression profile in any individual dataset, it did score highest in the meta-analysis. Several other top-scoring meta-outliers localize within 1 Mb of ERBB2 on chromosome 17q. As expected from the past observation that ERBB2 and genomic neighbors are coamplified and coexpressed in breast cancer (10, 11), we observed a clear coexpression pattern of the 17q meta-outliers (Fig. S2*B*).

The next most consistently scoring outlier, excluding ERBB2 and genomic neighbors, was AGTR1, the gene encoding angiotensin II receptor type I, which is the target of the antihypertensive drug losartan (12) and has previously been linked to cancer (12–17) and cancer-related signaling pathways (18, 19). AGTR1 was called an outlier in 15 of 22 datasets (68%; $P = 2.0E\text{-}18$). The microarray data clearly indicated that AGTR1 is highly overexpressed in a subset of tumors relative to normal tissue (Fig. 2*A*) and that high overexpression occurs exclusively in a subset of estrogen receptor-positive (ER^+) tumors (Fig. 2*C*). Furthermore, a coexpression analysis of AGTR1 and ERBB2 revealed a mutually exclusive relationship, with breast tumors overexpressing ERBB2 or AGTR1, but never both (Fig. 2 *B* and *D*). Additional evidence for the marked overexpression of AGTR1 in 10–20% of breast tumors, specifically ER^+, $ERBB2^-$ breast tumors, is presented in *SI Materials and Methods* (Figs. S3 and S4). AGTR1 overexpression was not significantly associated with 5-year recurrence-free survival in ER^+, $ERBB2^-$ breast cancer across 2 independent datasets (Fig. S5). We validated and quantified AGTR1 overexpression by quantitative RT-PCR in formalin-fixed, paraffin-embedded tissue from normal breast, primary breast cancer, and metastatic breast cancer. Consistent with the microarray data, we found AGTR1 to be more than 20-fold overexpressed in 7 of 45 tumors (15.5%) and more than 100-fold overexpressed in 2 primary tumors and 1 metastatic tumor (Fig. 2*E*).

Given the remarkable overexpression of AGTR1 in tumor subsets, we investigated potential mechanisms by which AGTR1 becomes overexpressed. First, using Oncomine, we examined AGTR1 coexpression data from 5 independent datasets, and in each case we found no more than one additional gene correlated with AGTR1 ($R > 0.5$), providing preliminary evidence that AGTR1 is not regulated as part of a larger transcriptional program. Second, we examined AGTR1 overexpression in the context of genes that neighbor AGTR1 on chromosome 3q. Unlike ERBB2, AGTR1 did not display any correlated expression with genomic neighbors (Fig. S6).

Next, we performed FISH on tissue microarrays to test the AGTR1 locus for gene rearrangement or DNA copy number aberration. Using a split probe strategy (8), we found that 5′ and 3′

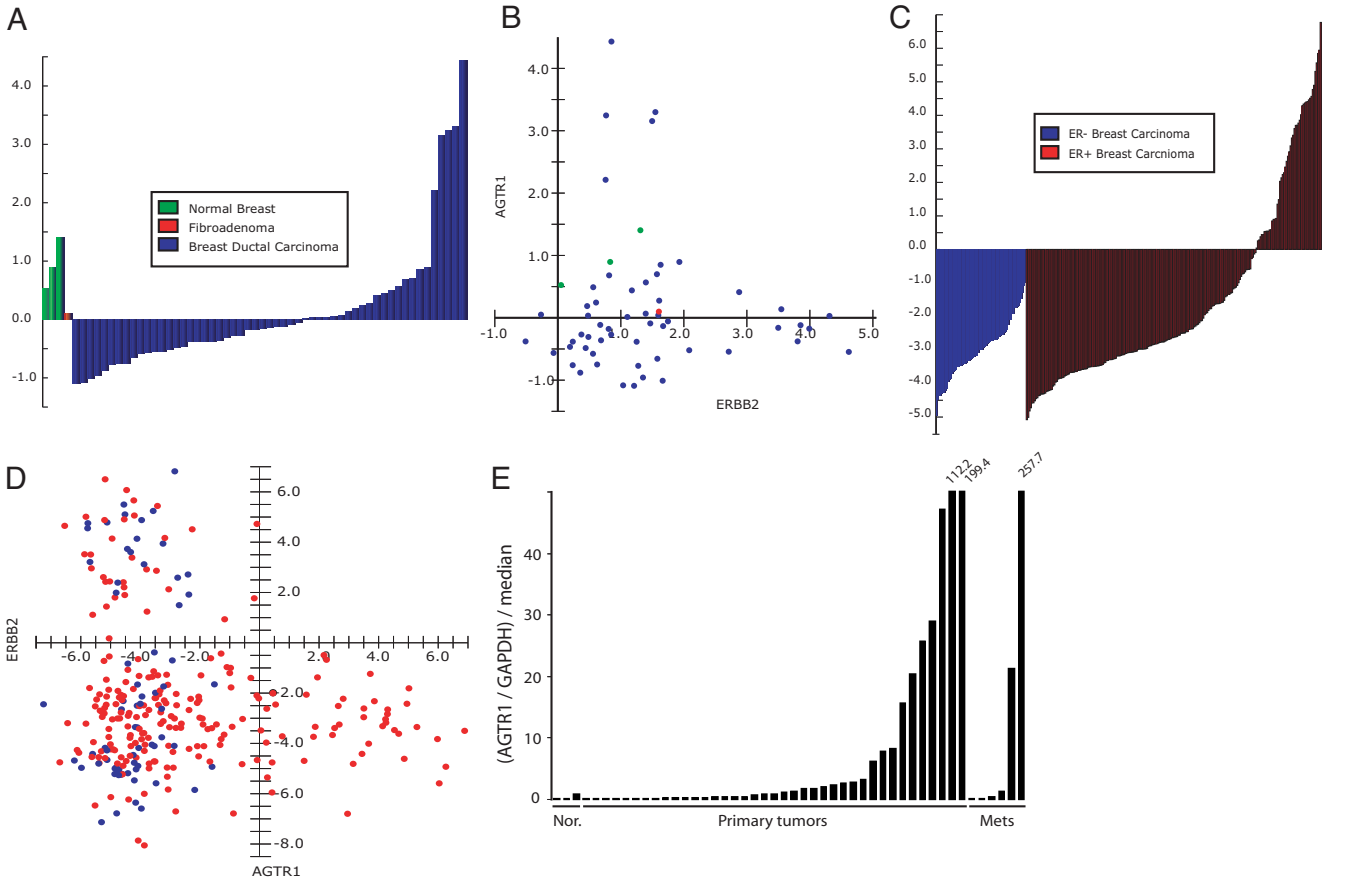

Fig. 2. AGTR1 outlier expression in breast cancer. (*A*) AGTR1 expression profile in the Perou et al. (29) cDNA microarray dataset ($n = 55$). (*B*) In the same dataset, AGTR1 expression vs. ERBB2 expression. (*C*) AGTR1 expression profile in the van de Vijver et al. (30) oligonucleotide dataset, segregated by ER status ($n = 295$). (*D*) AGTR1 expression vs. ERBB2 expression in the same dataset. (*E*) AGTR1 expression by quantitative RT-PCR in formalin-fixed, paraffin-embedded tissue. Expression of AGTR1 was assessed in 3 normal breast tissue specimens, 36 primary breast tumor specimens, and 9 metastatic breast cancer specimens. Expression levels were normalized to GAPDH expression and then scaled by the median AGTR/GADPH ratio.

AGTR1 probes never demonstrated consistent split signals, and thus concluded that rearrangement of the AGTR1 locus is not involved in AGTR1 overexpression. AGTR1 copy number was also evaluated in 112 breast carcinoma cases. Definitive copy number gain [locus/control (L/C) $>$ 1.5] was observed in 7 of 112 cases (6.25%), of which 6 were invasive ductal carcinoma and 1 was ductal carcinoma in situ (Fig. 3 *A* and *B*). To study the association between DNA copy number and overexpression, we identified available cases for qRT-PCR analysis, including 14 cases with no gain (L/C $<$ 1.2), 3 cases with questionable gain (1.2 $<$ L/C $<$ 1.5), and 4 cases with definitive DNA copy number gain (L/C $>$ 1.5). We observed a significant concordance between high AGTR1 expression and definitive copy number gain ($P = 0.006$; Fig. 3*C*). All 4 cases tested with definitive copy number gain also had high AGTR1 expression; however, high expression was also observed in 3 of 17 cases without definitive copy number gain. Thus, in this small sample set, copy number gain was always associated with overexpression, but overexpression also occurred without copy number gain.

To study the function of AGTR1 overexpression in breast epithelial cells, we generated an adenovirus construct expressing AGTR1. Human mammary epithelial cells (H16N2 and HME) were infected with AGTR1-expressing virus or control LacZ-expressing virus and cultured in serum-free media (Fig. S7). We assayed AGTR1-overexpressing cells and control cells for cell proliferation and invasion both in serum-free media and upon stimulation with angiotensin II (AT), the ligand of AGTR1. Overexpression of AGTR1 alone or in combination with AT did not affect cell proliferation. However, in both cell lines, we did observe that overexpression of AGTR1 with AT stimulation did significantly promote cell invasion in a reconstituted basement membrane invasion chamber assay (Fig. 4 *A* and *B*). The control experiment, in which the LacZ gene was transfected, did not exhibit increased invasion with AT stimulation. Importantly, AGTR1 and AT-mediated invasion was attenuated in a dose-dependent manner with inclusion of the AGTR1 blocker, losartan. Losartan had no effect on the LacZ-transfected cells or the AGTR1-transfected cells not stimulated with AT (Fig. 4*B*). To confirm that losartan inhibition of invasion is specific to AGTR1 transfection, we also infected H16N2 and HME cells with EZH2-expressing adenovirus, a gene known to induce invasion and, as expected, found that EZH2-mediated invasion was not attenuated by losartan treatment (Fig. S8). Thus, in 2 benign breast epithelial cell lines, AGTR1 overexpression in the presence of AT led to a markedly invasive tumorigenic phenotype, which is specifically reversed by treatment with losartan. We also tested the AGTR1-overexpressing mammary epithelial cells for activation of the MAPK and PI3K pathways, as measured by ERK phosphorylation and AKT phosphorylation, respectively. We found that AGTR1 overexpression combined with AT stimulation did increase ERK phosphorylation but not AKT phosphorylation. Losartan treatment (10 μM) inhibited the AT-stimulated increase in ERK phosphorylation (Fig. S9).

Next, we identified and tested a panel of breast cancer cell lines with endogenous AGTR1 overexpression. By using Oncomine (20), we identified 4 breast cancer cell lines with validated AGTR1

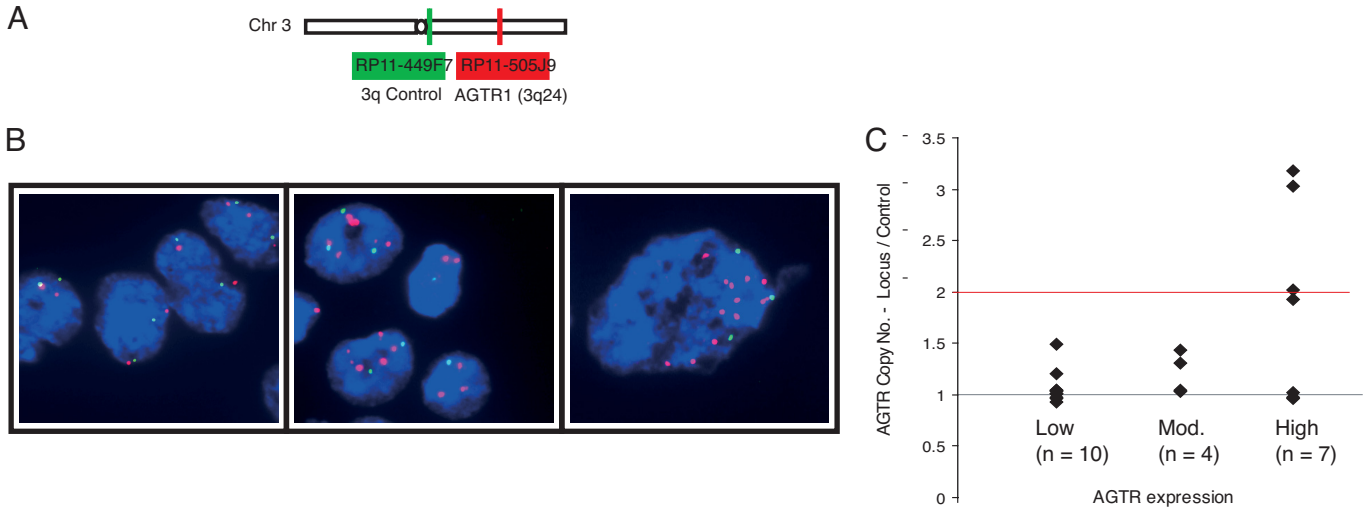

Fig. 3. Copy number analysis of the AGTR1 locus. (*A*) A schematic of probes used for FISH analysis. (*B*) Representative image from FISH analysis. *Left* is taken from a representative negative case. *Middle* and *Right* are images from a representative case with definitive copy number gain of AGTR1. Red signal is the AGTR1 locus probe, and green signal is the probe near the chromosome 3 centromere. (*C*) Association of AGTR1 overexpression with copy number gain. Three expression bins were defined based on AGTR1/GAPDH ratios: low (<1.0), moderate (1.0–2.0), and high (>2.0).

overexpression and 3 breast cancer cell lines with little or no expression of AGTR1 (Fig. S10). As an additional negative control, we also included the highly invasive prostate cancer cell line DU145, which has low expression of AGTR1. By using the reconstituted basement membrane invasion chamber assay, we tested the cell line panel with and without 1 μM AT and losartan. In each of the 4 AGTR1-overexpressing cell lines, we observed an increase in invasion upon stimulation with 1 μM AT, which was reversible by addition of losartan, whereas none of the 3 breast cancer cell lines with low AGTR1 expression, nor DU145, showed an increase in invasion upon 1 μM AT stimulation (Fig. 4*C*). Thus, we confirmed that our ectopic AGTR1 overexpression results can be generalized to breast cancer cells with endogenous overexpression but not those with low expression, and that losartan-mediated decrease in invasion is specific to invasion related to AT stimulation and AGTR1 overexpression.

Next, we stably transfected AGTR1 into MCF7 human breast cancer cells and performed mouse xenograft studies. We implanted MCF7-AGTR1 cells or MCF7-GUS control cells into the mammary fat pad of nude mice and treated animals with 90 mg/kg losartan per day or vehicle control. We studied the impact of losartan on tumor growth at 2 weeks and 8 weeks. Ten mice were studied in each group: MCF7-AGTR1 plus saline, MCF7-AGTR1 plus losartan, MCF7-GUS plus saline, and MCF7-GUS plus losartan. MCF7-AGTR1 tumors did not display increased growth at 2 weeks or 8 weeks relative to MCF7-GUS control tumors. Losartan treatment did, however, significantly reduce early and late tumor growth in MCF7-AGTR1-implanted mice but had no effect on tumor growth in MCF7-GUS control-implanted mice. At 2 weeks after implantation, the median tumor size of MCF7-AGTR1 tumors treated with losartan was 20% smaller than MCF7-AGTR1 tumors treated with vehicle control (P = 1.4E-4; Fig. 5*A*). On the contrary, there was no significant change in tumor size at 2 weeks in MCF7-GUS tumors treated with losartan relative to vehicle control (P = 0.67). Similarly, at 8 weeks, median tumor size of MCF7-AGTR1 tumors treated with losartan was 31% smaller than those treated with control (P = 0.016; Fig. 5*B*). Again, no significant change in median tumor size of MCF7-GUS tumors was observed upon losartan treatment (P = 0.24). In summary, although AGTR1 transfection into MCF7 breast cancer cells did not increase tumor size, it did significantly sensitize tumors to growth inhibition with losartan treatment.

Discussion

In summary, we performed a large-scale meta-analysis of outlier expression profiles across several large cohorts of breast tumors. Our analysis prioritized genes with marked overexpression in subsets of tumors. This approach correctly prioritized the prototypical breast cancer oncogene and drug target ERBB2. In addition, several new genes were identified, demonstrating consistent and dramatic overexpression in tumor subsets. We suspect that our analysis has uncovered a new crop of potentially important breast cancer genes.

AGTR1, the angiotensin II receptor, was found to be one of the most highly overexpressed genes in 10–20% of breast cancers across independent breast cancer microarray studies. This has potential clinical importance because AGTR1 is antagonized by commonly prescribed antihypertensive agents (12), such as losartan, which have been shown to have antitumorigenic effects in model systems (12–17). Interestingly, AGTR1 always displayed high overexpression in ER-positive, ERBB2-negative tumors, potentially providing insights into the selective pressures governing AGTR1 activation in breast cancer. Contrary to expectation, ER in fact down-regulates the AGTR1 transcript via cytosolic mRNA-binding proteins (21). Thus, we hypothesize that the paradoxical marked overexpression of AGTR1 in a subset of ER^+ breast tumors may be the result of a genetic aberration that put the AGTR1 transcript under the positive control of the ER. Based on the mutually exclusive expression pattern with ERBB2 and the reported overlapping downstream pathways affected by AGTR1 and ERBB2, we suspect that AGTR1 activation and ERBB2 activation may represent alternative but functionally related events in tumorigenesis. Our AGTR1 transfection experiments in HME cells confirmed that ERK phosphorylation, a MAPK pathway readout, increases upon angiotensin stimulation.

We applied computational and experimental strategies to uncover mechanisms for AGTR1 overexpression. Coexpression analysis revealed that AGTR1 is not likely to be part of a larger transcriptional program, because other genes were not found to be highly coexpressed with AGTR1. FISH analysis demonstrated that chromosomal rearrangements do not occur at the AGTR1 locus, making gene fusions an unlikely cause of overexpression. DNA copy number analysis did identify a small fraction (6.5%) of breast tumors with increased copy number at the AGTR1 locus, and copy number gain occurred only in cases with overexpression. However,

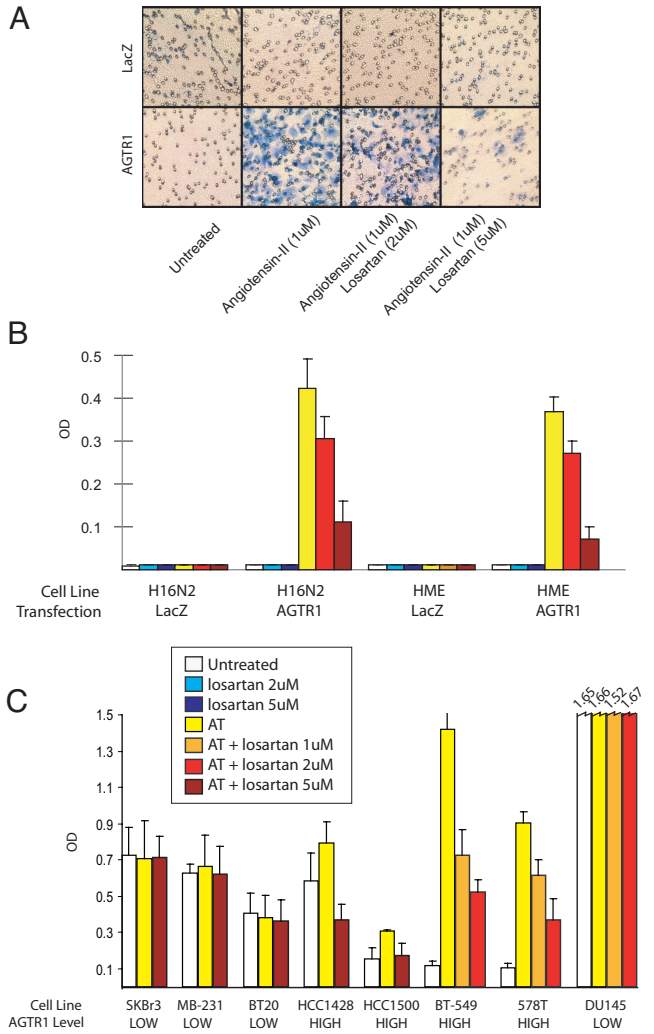

Fig. 4. AGTR1 overexpression and analysis of angiotensin II (AT) and losartan effects on cell invasion. (*A*) Matrigel invasion assays of H16N2 cells infected with adenovirus expressing AGTR1 or LacZ. Cells were cultured in serum-free media and were pretreated with and without AT and losartan. Similar results were observed for HME cells. (*B*) Colorimetry readout of invasion assays from transfection experiments. LacZ- or AGTR1-expressing adenovirus was infected into H16N2 and HME immortalized mammary epithelial cells, and cells were treated with or without 1 μM AT and losartan. Because of absent baseline invasion, the optical density (OD) measurements were background subtracted, and values below 0.01 were set to 0.01. (*C*) Colorimetry readout of invasion assays from a panel of cancer cell lines. Seven breast cancer cell lines and a prostate cancer cell line, DU145, were examined for invasion after treatment with or without 1 μM AT and losartan. AGTR1 expression levels are indicated and were obtained from published microarray data and qRT-PCR analysis (Fig. S7). The quantification of invasion was done as described in *B*.

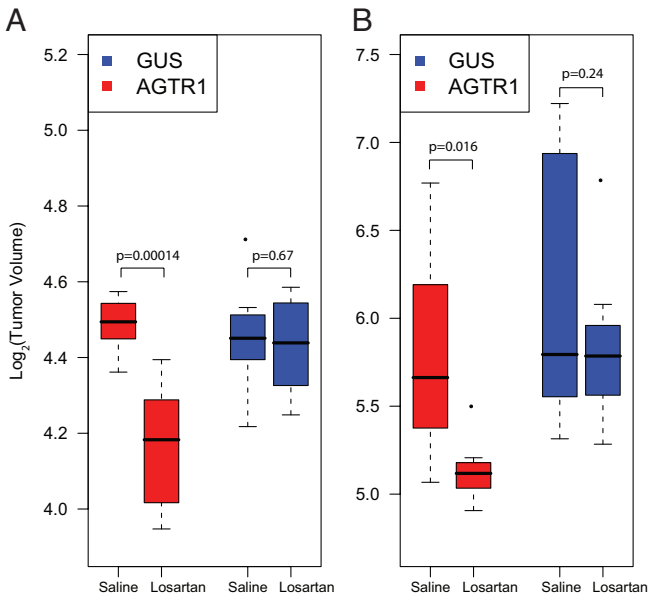

Fig. 5. Effect of losartan treatment on AGTR1- or GUS-overexpressing MCF7 cell xenografts. Female BALB/C nu/nu mice were implanted with 2.5 $\times$ 10^6 stable MCF7 cells overexpressing AGTR1 or GUS resuspended in 100 μL of saline with 20% Matrigel into the mammary fat pad of anesthetized mice. Mice from both groups: MCF7-AGTR1 or MCF7-GUS (n = 10 for each group) were treated every day with losartan (90 mg/kg body weight) or vehicle control. All animals were monitored at weekly intervals for tumor growth, and tumor sizes were recorded using the formula (π/6) ($L \times W^2$), where L = length of tumor and W = width. Box plots of $\log_2$ tumor volumes are shown. P values from 2-sided Student's t tests indicate statistical significance. (*A*) Xenograft tumor size at 2 weeks. (*B*) Xenograft tumor size at 8 weeks.

some overexpressing cases did not have copy number gain, and the level of copy number gain observed in positive cases was not proportional to the degree of overexpression observed. Thus, we suspect that copy number gain contributes to overexpression in some cases but is not likely to be the predominant mechanism. Future studies to investigate the mechanism of AGTR1 overexpression should include high-resolution array comparative genomic hybridization and sequencing of the AGTR1 locus.

Regardless of the mechanism, AGTR1 undergoes profound deregulation in a subset of breast cancers, and our in vitro and in vivo studies demonstrate a functional role for AGTR1 overexpression in breast cancer and, more importantly, the potential for targeting AGTR1$^+$ breast tumors with an available therapy. Past work has shown that in breast cancer cell lines, angiotensin II stimulation evokes an invasive phenotype, which is inhibited by losartan treatment (22). Furthermore, it was demonstrated that the increase in invasion is coincident with decreased expression of integrins, possibly via protein kinase C signaling. Although these observations were made in transformed breast cancer cells naturally expressing AGTR1, our work shows that activated AGTR1 pathway, by way of artificial AGTR1 overexpression, in normal breast epithelial cells is sufficient to activate an invasive phenotype, suggesting that this pathway may be especially important in breast tumors with high overexpression. Furthermore, we studied a panel of cell lines with either high or low levels of AGTR1 and showed a clear correlation between AT-mediated invasion and level of AGTR1 expression.

Our in vivo data provide further evidence that losartan may be a viable therapy for women with AGTR1-overexpressing breast tumors. Breast cancer xenografts overexpressing AGTR1 were differentially sensitive to losartan treatment, demonstrating a 30% reduction in growth at 8 weeks, whereas control xenografts had no reductin in tumor size. It is interesting that MCF7-AGTR1 xenografts did not display increased growth relative to MCF7 control xenografts, but they did display a significantly increased losartan effect. This suggests that AGTR1 does not provide an additive growth signal to MCF7 cells, which do harbor an activating PI3K mutation. We suspect that the stable transfection of AGTR1 reprogrammed MCF7 cells to be at least partially dependent on AGTR1 as a growth or survival signal; hence, the differential response to losartan. We anticipate that de novo AGTR1-positive primary tumors may be even more dependent on the AGTR1 signal, and thus more sensitive to inhibition.

Interestingly, past studies have linked polymorphisms in the angiotensin pathway with breast cancer incidence (23, 24), documenting a significant increase in breast cancer incidence in

women with the D/D angiotensin-converting enzyme (ACE) allele, which is associated with increased circulating ACE levels, and thus increased levels of angiotensin II, the ligand for AGTR1. Other studies have examined the relationship between antihypertensive therapy (AHT), which often involves modulation of the angiotensin axis, and breast cancer incidence. The largest of such studies did not observe a significant relationship (25); however, the study examined a variety of AHT modalities and was likely not powered to detect a small change incidence that might be expected from a response only in the AGTR1$^+$ subpopulation.

In summary, this study provides a rationale for a clinical trial that includes losartan in the treatment of breast cancer patients with tumors positive for AGTR1. We demonstrated that AGTR1 transcript levels and DNA copy number can be effectively measured from formalin-fixed, paraffin-embedded tissue specimens, thus enabling the identification of the appropriate patient population.

Materials and Methods

MetaCOPA Analysis. COPA analysis was performed on 31 breast cancer gene expression datasets in Oncomine (www.oncomine.org) as described previously (8). Genes scoring in the top 1% of COPA scores at any of the 3 percentile cutoffs (75th, 90th, and 95th) were deemed outliers in their respective datasets. Meta-outliers were defined as genes deemed outliers in a significant fraction ($P < 1E-5$) of datasets as assessed by the binomial distribution. Analysis details are provided in *SI Materials and Methods*.

Quantitative PCR (QPCR). QPCR was performed by using SYBR Green dye on an Applied Biosystems 7300 Real Time PCR system (Applied Biosystems) essentially as described previously (8). Details and primer sequences are available in *SI Materials and Methods*.

AGTR1 Transfection. The benign human mammary epithelial cells HME and H16N2 were transfected with AGTR1-expressing adenovirus and assayed for cell invasion with or without losartan and angiotensin II treatment. Details are available in *SI Materials and Methods*.

Cell Invasion Assay. Breast cell lines BT-549, Hs578T, HME, H16N2, HCC1528, HCC1500 and prostate carcinoma line DU145 were assayed for cell invasion with or without losartan and angiotensin II treatment using Matrigel invasion chambers. Details are available in *SI Materials and Methods*.

AGTR1 Amplification Assessment. A breast cancer tissue microarray containing 311 cases of invasive breast cancer was tested for AGTR1 locus amplification by flourscence in situ hybridization. Details are available in *SI Materials and Methods*.

Mammary Fat Pad Xenograft Model. Balb/C nu/nu mice were implanted with MCF7 cells stably overexpressing AGTR1 or Gus and then treated daily with losartan vehicle control. Details are available in *SI Materials and Methods*.

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