Insulin-Like Growth Factor-I Activates Gene Transcription Programs Strongly Associated With Poor Breast Cancer Prognosis

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A B S T R A C T

Purpose

Substantial evidence implicates insulin-like growth factor-I (IGF-I) signaling in the development and progression of breast cancer. To more clearly elucidate the role of IGF in human breast cancer, we identified and then examined gene expression patterns of IGF-I-treated breast cancer cells.

Methods

MCF-7 cells were stimulated with IGF-I for 3 or 24 hours and were profiled for greater than 22,000 RNA transcripts. We defined an IGF-I signature pattern of more than 800 genes that were up- or downregulated at both time points. The gene signature was examined in clinical breast tumors and in experimental models that represented other oncogenic pathways. The signature was correlated with clinical and pathologic variables and with patient outcome.

Results

IGF-I caused temporal changes in gene expression that were strongly associated with cell proliferation, metabolism, and DNA repair. Genes with early and sustained regulation by IGF-I were highly enriched for transcriptional targets of the estrogen receptor (ER), Ras/extracellular signal-related kinase 1/2, and phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathways. In three large, independent data sets of profiled human breast tumors, the IGF-I signature was manifested in the majority of ER-negative breast tumors and in a subset (approximately 25%) of ER-positive breast tumors. Patients who had tumors that manifested the IGF-I signature (including patients who did not receive adjuvant therapy) had a shorter time to a poor outcome event. The IGF gene signature was highly correlated with numerous poor prognostic factors and was one of the strongest indicators of disease outcome.

Conclusion

Transcriptional targets of IGF-I represent pathways of increased aggressiveness and possibly hormone independence in clinical breast cancers.

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INTRODUCTION

Substantial evidence implicates insulin-like growth factor (IGF) signaling in the development and progression of many cancers, including breast cancer.¹ High serum IGF-I levels predict an increased risk of breast cancer,² and antiestrogens that are effective in the treatment and prevention of breast cancer reduce serum IGF-I levels.³ IGF-I receptor (IGF-IR) is hyperactive and overexpressed in many breast cancers.⁴ Preclinical data have validated IGF-IR as a therapeutic target, and several IGF-IR inhibitors have recently entered clinical trials.⁵

After IGF-I binds to its receptor, it activates a cascade of downstream signaling events. Although great progress has been made regarding the mechanism of IGF-IR signaling at the immediate level of protein phosphorylation cascades,¹ relatively little is known regarding transcriptional targets of the IGF-I pathway. Analysis of these targets may shed light on the pathway's role in breast cancer and may provide biomarkers of IGF-I action. In this study, we used gene expression profiling to identify IGF-I-regulated genes in breast cancer cells. We identified a set of more than 800 genes in which expression was increased or decreased by IGF-I

(ie, an IGF-I gene signature) that is present in a significant proportion of human breast cancers and that indicates a poor prognosis.

METHODS

Cell Line and Culture Conditions

MCF-7L ER-positive breast cancer cells were obtained originally from American Type Culture Collection and have been extensively described previously. Cells were routinely maintained in Dulbecco's Modified Eagle's Medium + 5% fetal bovine serum. Cells were plated in 10-cm dishes and then switched on the next day to serum-free medium. After an overnight incubation, cells were stimulated in triplicate with or without IGF-I (8 nmol/L or 50 ng/mL; GroPep, Adelaide, Australia) for 3 or 24 hours (serum-free medium 3-hours group was in duplicate) as well as with or without 1 nmol/L estradiol. Cells were then lysed, and total RNA was isolated by using the Qiagen midi-prep kit (Valencia, CA) according to the manufacturer's instructions.

Affymetrix Microarray Analysis

cDNA synthesis, cRNA labeling, and array hybridization were performed as previously described²⁵ by using Affymetrix U133A 2.0 gene chips (Santa Clara, CA). Array data has been deposited in the public Gene Expression Omnibus database, accession GSE7561. Arrays were processed and normalized by using the dChip software (<http://biosun1.harvard.edu/complab/dchip/>).²⁶ Expression values were visualized as color maps by using the Cluster and Java TreeView software (<http://rana.lbl.gov/EisenSoftware.htm>).^{27,28} Data were analyzed through the use of Ingenuity Pathways Analysis (Ingenuity Systems, Redwood City, CA).

RESULTS

Global Gene Transcription Patterns Initiated by IGF-I Treatment of MCF-7 Cells

ER-positive and IGF-responsive MCF-7 human breast cancer cells were starved overnight in serum-free medium (SFM) and were then stimulated with or without IGF-I for 3 or 24 hours. RNA was isolated, and gene expression array profiles were analyzed with GS (gene expression data deposited at the Gene Expression Omnibus database, GSE7561). After filtering the genes (Appendix, online only), 13,731 RNA transcripts (8,496 unique, named genes) were represented. A comparison of IGF-I with SFM treatment revealed 2,154 transcripts (1,745 unique genes) that showed significant changes ($P < .01$, fold change > 1.5) at 3 or 24 hours or both. By hierarchical clustering, these genes separated into two approximately equal-sized groups of up- and downregulated genes (Appendix Fig A1, online only).

Genes that were increased or decreased by IGF-I included some expected targets but also many novel targets. For example, we have reported previously that IGF-I can repress progesterone receptor (PR) mRNA expression via phosphatidylinositol 3-kinase (PI3K)/Akt inhibition of the PR promoter⁶ and that PR levels in our data set were strongly downregulated ($P < .001$) at 3 hours. Several other genes in the insulin/IGF pathway were regulated, including *INSIG1*, *IGF2BP2*, *IRS1*, and *IRS2*.

We found significant concordance between genes that we identified as being regulated by IGF-I stimulation of MCF-7 cells and genes from a recently published study of MCF-7 cells that were stably overexpressing IGF-I⁷. Of the 24 genes reported as upregulated by IGF-I⁷, 15 likewise were increased ($P < .05$) by IGF-I at 24

hours in our data set ($P < .000001$, one-sided Fisher exact); of five genes previously downregulated by IGF-I, three likewise were decreased in our data set (Appendix).

To examine the biologic function of IGF-I-regulated genes, we performed ingenuity pathway analysis (IPA). IPA indicated significant representation within IGF-regulated genes related to the cell cycle and to cell growth and proliferation (Appendix Figs A2A and A2B, online only), which was consistent with the status of IGF-I as one of the most potent mitogens for human breast cancer cells. Indeed, IGF-I altered mRNA levels of numerous cyclins (*CCNA1*, *A2*, *B1*, *D3*, *E1*, *E2*, *F*, *G1*, and *G2*), cell-division cycle proteins (*CDC14A*, *14B*, *2*, *A3*, *A4*, *A6*, *A8*, *20*, *25A*, *25B*, *25C*; *2L6*, *42EP1*, and *45L*), and cyclin-dependent kinases (*CDK2*, *AP2*, *N1A*, *N1B*, *N2C*, and *N3*). IPA analysis revealed temporal changes in IGF-I-regulated genes that affected different cellular pathways and functions. For example, 3 hours of IGF-I treatment showed regulation of G1/S checkpoint genes (Appendix Fig A3A, online only), whereas there was an effect on G2/M DNA damage checkpoint genes after 24 hours (Appendix Fig A3B). An example of IGF-I regulation of a gene expression network associated with G2/M DNA damage checkpoint regulation is shown (Appendix Fig A4, online only). IPA canonical pathway analysis revealed regulation of insulin and IGF-I signaling, as might be predicted; however, numerous other growth factor signaling pathways were associated, including epidermal growth factor, fibroblast growth factor, and vascular endothelial growth factor.

To more clearly articulate temporal changes in gene expression, we performed a supervised clustering analysis that compared SFM and IGF-I at 3 or 24 hours or both. Each transcript was assigned to an expression pattern of time-dependent induction or repression (Fig 1A). The global transcription patterns of IGF-I signaling showed temporal complexity; different genes showed induction or repression at different time points. Selected enriched Gene Ontology (GO) annotation terms for each gene cluster are indicated in Figure 1A.

Genes With Early and Sustained Regulation by IGF Enriched for Transcriptional Targets of the Ras/Extracellular Signal-Related Kinase 1/2 and PI3K/Akt/Mammalian Target of Rapamycin Pathways

IGF-I is known to signal via rapid phosphorylation and activation of the Grb2/sos/Ras/ extracellular signal-related kinase 1/2 (ERK1/2) and the PI3K/Akt/mammalian target of rapamycin (mTOR) signaling cascades,¹ which are generic to many growth factor signaling pathways. Surprisingly, although IGF-I activates these protein cascades at the level of phosphorylation, alterations in the mRNA levels of many of the same proteins were noted as well. Thus, IGF-I altered levels of numerous PI3K family members (*PIK3C3*, *CA*, *R1*, *R3*, and *R4*), mitogen activated protein kinase (MAPK) family members (*MAP2K2*, *3*, *5*, *6*, *7*, *8*; *MAPK9*; and *MAPKAPK5*), and several dual-specificity phosphatases of MAPKs (*DUSP2*, *4*, *5*, *6*, *8*, *10*, *12*, and *13*).

We continued to examine pathways activated by IGF-I by using public array data sets that defined downstream transcriptional targets of specific oncogenic pathways.⁸⁻¹⁰ We focused on a set of 1,084 RNA transcripts (903 unique, named genes) that were up- or downregulated by IGF at 3 and 24 hours ($P < .01$; fold change > 1.5 at either time point; $P < .05$ at the other time point). We reasoned that these genes represented early and sustained targets of IGF signaling

Fig 1. Global gene transcription patterns initiated by insulin-like growth factor (IGF) treatment in vitro. Affymetrix profiles were taken of MCF-7 cells in serum-free medium with or without the presence of IGF-I (8 nmol/L) at 3 and 24 hours. (A) Supervised clustering of 2,154 RNA transcripts that showed up- or downregulation by IGF at 3 or 24 hours ($P < .01$, fold change > 1.5). For indicated clusters, representative enriched Gene Ontology annotation terms ($P < .0005$) are given (including the number of unique genes in the cluster v the number represented on the Affymetrix array). (B) Genes with early and sustained regulation by IGF from 3 through 24 hours. Alongside the IGF treatment profile data set are the corresponding expression patterns in the profile data sets from Bild et al⁸ (Duke Oncogene Activation) of breast cells with activation of indicated oncogenes and from Lamb et al⁹ (Broad Connectivity Map) of cultured cells treated by 184 different small-molecule inhibitors. (Indicated profiles are highlighted). (C) An IGF gene signature of genes from (B) with proliferation-associated genes removed. Corresponding patterns of genes in estrogen-stimulated and in oncogene-activated University of Michigan (MI Oncogene activation¹⁰) MCF-7 cells are shown.

(whereas genes regulated at 24 hours but not at 3 hours represented more secondary effects, such as cell proliferation).

When we viewed the expression patterns of our early and sustained targets of IGFs in one profile data set⁸ of breast cells activated with various oncogenes, we found widespread similarities between our IGF-regulated genes and a Ras signature pattern (Fig 1B). Notably, the *HRAS* gene itself showed upregulation by IGF in the array data (Fig 1B).

We compared our early and sustained targets of IGF (Fig 1B) with a public database (<http://www.broad.mit.edu/cmap>) of gene expression profiles of cultured cells (mostly MCF-7 cells) that were treated with 164 different small molecule inhibitors, called the connectivity map (CMap).⁹ CMap analysis indicated that our IGF gene signature showed an inverse correlation with the expression patterns induced by the PI3K inhibitors LY-294002 and wortmannin, by the mTOR inhibitor rapamycin, or by the histone deacetylase (HDAC) inhibitor trichostatin A (Fig 1B). A significant number of 179 of the 422 genes upregulated by IGF were also downregulated by inhibition of PI3K; genes downregulated by IGF likewise tended to be upregulated by inhibition of PI3K (Appendix).

Similarities Among the Transcriptional Programs of IGF, Estrogen, and ERBB Signaling Pathways

Numerous studies have detailed extensive cross-talk between the IGF signaling pathway and two major growth regulators in breast cancer, namely ER and epidermal growth factor receptor/ERBB.¹¹ Therefore, we sought to examine the level of similarity among these signaling cascades. As IGF-IR, ER, and ERBBs all stimulate cell proliferation, we first removed from additional consideration 108 transcripts (89 genes) from the 1,084 transcripts (903 genes) defined as early and sustained targets of IGF (Fig 1B), that were either annotated as cell cycle by GO or were found experimentally to be correlated with cell cycle progression¹² so that any associations made between the

remaining genes would not be caused by generic cell proliferation effects. We then compared our IGF gene signature of 976 transcripts (814 genes) with gene expression signatures from MCF-7 cells that were stimulated with estrogen or that had activated ERBB signaling¹⁰ (Fig 1C). To compare with estrogen, we profiled estrogen-treated MCF-7 cells by using the exact same conditions as for IGF-I (Appendix). We found extensive overlap (approximately 31% of genes) between the IGF and estrogen signatures (Fig 1C and Appendix). Additional data supported this observation, as we found a virtually identical number of genes (approximately 31%) that overlapped between our IGF signature and another public estrogen signature.¹³ However, despite this large overlap and similarity between estrogen and IGF signatures, many well-known targets of estrogen signaling, such as *PGR*, *NPY1R*, and *IRS1*, were not among the IGF-upregulated genes and were, in fact, downregulated by IGF.

We also found significant, albeit minor, overlap between the IGF upregulated genes and sets of genes upregulated in MCF-7 cells that had overexpression of ligand-activated EGFR or ERBB2.¹⁰ The IGF signature was partly distinct from an EGFR/ERBB2 signature, because many genes showed completely discordant regulation between IGF and EGFR/ERBB2, and the majority of IGF-regulated genes were not altered by expression of EGFR/ERBB2 (Fig 1C; Appendix Fig A5A, online only).

IGF-Induced Gene Expression Patterns Associated With ER-Negative Breast Cancer

We examined the expression patterns of the genes induced or repressed by IGF-I at both 3 and 24 hours (IGF gene signature; Fig 1C) in human breast tumors to ascertain the possible clinical context and relevance. As stated previously, the IGF gene signature has transcripts removed that were involved in cell cycle progression so that any associations made between the remaining genes and the clinical

Fig 2. An insulin-like growth factor (IGF) gene signature in clinical breast tumors is associated with estrogen receptor (ER)-negative status and basal/luminal "B" tumor subtypes. (A) Genes in the IGF gene signature shown in Figure 1C were examined in three published profile data sets of clinical breast tumors from van de Vijver,¹⁴ Wang,¹⁵ and Miller.¹⁶ Patterns for genes that were upregulated in the IGF gene signature are separate from the patterns for genes that were downregulated. Within ER-positive and ER-negative subtypes, tumors are ordered from those that have low similarity with the IGF patterns to those with high similarity; tumors with significant positive correlations ($P < .01$) are bracketed. Red bars along the bottom indicate poor outcome events in patients (gray; data not available). (B) As in (A), for the Sorlie tumor data set.¹⁷

Fig 3. Gene expression patterns associated with insulin-like growth factor (IGF) signaling are correlated with poor prognosis. Kaplan-Meier analysis of three tumor profile data sets (A and D from Van de Vijver et al; B and E from Wang et al; and C and F from Miller et al) comparing the differences in risk among three groups of patients when (A to C) all tumors were considered or when (D to F) only those classified as ER-positive were considered. Yellow line, tumors that have high similarity (ie, correlation; $P < .01$) to the pattern of up- and downregulation by IGF observed in vitro in Figure 1C; blue line, tumors that have low similarity (ie, anticorrelation; $P < .01$) to the IGF pattern; and gray line, tumors neither similar nor dissimilar to the IGF pattern ($P > .01$). The log-rank test evaluated whether there are significant differences among any of the three groups. The univariate Cox test evaluated the association of the IGF activation score with patient outcome when the coefficient was treated as a continuous variable.

tumors would not be caused by generic cell proliferation effects (Appendix Fig A6A, online only). When we examined the levels of the genes in our signature in three independent expression profile data sets of clinical breast tumors from van de Vijver et al,¹⁴ Wang et al,¹⁵ and Miller et al,¹⁶ a striking correlation was observed (Fig 2A). Most of the ER-negative breast tumors showed high expression of the genes induced in vitro by IGF and low expression of the repressed genes, and this was highly consistent among the three independent data sets. In ER-positive tumors, approximately 25% (which had relatively lower levels of ER) showed a similar correlation with the IGF-I signature. Genes in our IGF gene signature appeared coordinately expressed in human breast tumors, even with rigorous testing for chance associations (Appendix).

We examined an additional clinical breast tumor profile data set from Sorlie et al,¹⁷ in which the profiles were subtyped on the basis of gene clustering. The IGF gene signature appeared activated in most of the basal tumors (associated with ER-negative/HER2-negative status), in most of the luminal B tumors (ER-positive with poor prognosis), and in a sizable subset of the ERBB2-positive tumors (ER-negative/HER2-positive), but not in the luminal A or normal breast-like tumors (Fig 2B).

Gene Signature Patterns of IGF Signaling Associated With Adverse Pathologic and Clinical Markers and With Poor Prognosis in Breast Cancer

We defined an IGF activation score for a given tumor as the Pearson correlation between the IGF gene signature pattern (by using 1 and -1 for up and down, respectively) and the gene expression values of the tumor. In the van de Vijver, Wang, and Miller data sets, the activation score was inversely correlated with mRNA levels (as measured on the array) of both ER and PR within the ER-positive tumors ($r < -0.3$, $P < .00001$; and $r < -0.18$, $P < .01$, respectively, Appendix Table A1). In the van de Vijver and Miller data sets, the scores were highly correlated with increasing grade ($r = 0.46$, $P < 5 \times 10^{-13}$; and $r = 0.46$, $P < 1 \times 10^{-12}$, respectively), tumor size ($r = 0.15$, $P < .02$; and $r = 0.23$, $P < .001$, respectively) and lymph nodes ($r = 0.20$, $P = .002$; and $r = 0.22$, $P = .002$, respectively) within ER-positive tumors in both data sets and were inversely correlated with age in the Miller data set ($r = -0.18$, $P < .01$; Appendix Table A1).

Given the association of the IGF gene signature with known poor prognostic markers, we examined the association of the signature with patient outcome. Kaplan-Meier curves and univariate Cox analysis

showed that patients with tumors that had high IGF activation scores had reduced disease-specific survival or metastasis-free survival compared with patients who had tumors that showed an inverse correlation with the IGF-I signature (Fig 3A). Interestingly, patients with tumors that had no significant correlation with the IGF-I signature ($P > .01$, Pearson) had an intermediate prognosis. As described above, the IGF gene signature showed activation predominantly in ER-negative breast cancer. As ER-negative breast cancer frequently shows poorer prognosis compared with ER-positive breast cancer,¹⁸ we examined the subset of ER-positive cancers and found the IGF gene signature to be an indicator of outcome there as well (Fig 3B).

The Wang data set represented patients who had small tumors, who were node-negative, and who received only local therapy, which thus indicated that the IGF gene signature reflects poor prognosis independent of treatment. The van de Vijver and Miller data sets both contained a proportion of patients who received adjuvant therapy. We examined the effect of the IGF gene signature in only the subset of 165 patients from the van de Vijver data set that did not receive hormone therapy or chemotherapy, and again we found that the IGF-I signature predicted poor outcome (Appendix Fig A7, online only). Therefore, the IGF-I signature is a highly significant indicator of poor prognosis.

In a multivariate Cox model, in which poor patient outcome was evaluated among tumors in relation to ER status, ER mRNA, PR mRNA, HER2 mRNA, age, grade, tumor size, and lymph node status, the IGF gene signature provided significant predictive power that was independent of the other clinical variables (Table 1; van de Vijver $P < .001$, Wang $P = .004$, and Miller $P = .006$).

Analysis of the Cox multivariate models with and without the IGF gene signature indicated that the gene signature replaced grade in the model (presumably caused by the high correlation of the signature with grade; Appendix Table A1). Importantly, the prognostic ability of the IGF signature was not a result of genes generic to proliferation, because a proliferation score derived from 763 genes in the cell cycle signature from Whitfield et al.¹² et al was prognostic ($P < .05$, all three data sets) in univariate Cox analysis, but the prognostic ability was lost in multivariate models that incorporated other clinical variables with or without the IGF signature activation score. (Appendix Table A2).

DISCUSSION

In this study, we experimentally defined a gene transcription signature of IGF-I signaling in human MCF-7 breast cancer cells. IGF-I altered levels of a large portion of the transcriptome (approximately 21%), with genes involved in cell growth, survival, metabolism, and biosynthesis. IGF-I-altered mRNA levels of numerous members of both the PI3K and ERK1/2 pathways and the IGF-I signature were enriched for transcriptional targets of PI3K/Akt/mTOR and Ras/MAPK pathways. The IGF gene signature was highly activated in ER-negative breast tumors but also in a sizeable subset (approximately 25%) of ER-positive tumors that had low levels of ER, which suggests that this transcriptional program may

Table 1. Multivariate Cox Proportional Analysis of Tumor Characteristics and IGF Gene Signature in Relation to the Likelihood of Poor Outcome

Variable	Data Sets*								
	van de Vijver ¹⁴			Wang ¹⁵			Miller ¹⁶		
	<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI
Analysis Without IGF Gene Signature									
Age at surgery	.35	0.76	0.42 to 1.37	—	—	—	.37	0.73	0.36 to 1.48
Clinical tumor size	.03	1.58	1.03 to 2.43	—	—	—	< .001	3.20	1.58 to 6.59
Tumor grade†	< .001	1.69	1.24 to 2.31	—	—	—	.26	1.32	0.81 to 2.15
Lymph node status	.61	0.90	0.60 to 1.36	—	—	—	< .001	2.79	1.54 to 5.07
ER status‡	.40	1.51	0.57 to 3.96	.83	1.08	0.55 to 2.09	.26	1.8	0.61 to 5.78
ER mRNA‡	.67	0.91	0.60 to 1.39	.31	1.16	0.87 to 1.55	.83	1.05	0.69 to 1.59
PR mRNA‡	.004	0.70	0.55 to 0.90	< .001	0.69	0.55 to 0.85	.49	0.89	0.63 to 1.25
HER2 mRNA‡	.39	1.08	0.90 to 1.30	.69	0.96	0.79 to 1.17	.07	1.28	0.97 to 1.70
Analysis With IGF Gene Signature									
Age at surgery	.37	0.77	0.43 to 1.38	—	—	—	.38	0.73	0.36 to 1.50
Clinical tumor size	.05	1.54	1.00 to 2.38	—	—	—	.004	2.83	1.38 to 5.78
Tumor grade†	.26	1.22	0.86 to 1.72	—	—	—	.76	0.92	0.54 to 1.58
Lymph node status	.19	0.76	0.50 to 1.15	—	—	—	< .001	3.04	1.66 to 5.54
ER status‡	.31	1.65	0.61 to 4.47	.62	1.19	0.60 to 2.34	.32	1.76	0.57 to 5.46
ER mRNA‡	.78	1.06	0.68 to 1.65	.04	1.38	1.01 to 1.89	.24	1.29	0.83 to 2.01
PR mRNA‡	.02	0.75	0.58 to 0.96	< .001	0.70	0.56 to 0.87	.54	0.90	0.64 to 1.27
HER2 mRNA‡	.21	1.12	0.93 to 1.35	.69	0.96	0.79 to 1.17	.08	1.27	0.97 to 1.65
IGF signature‡	< .001	1.86	1.36 to 2.53	.004	1.43	1.12 to 1.82	.006	1.81	1.17 to 2.79

NOTE. Age, size, lymph node status, and ER status were binary variables (0 for age < 50 years and 1 for an age ≥ 50 years; 0 for diameter ≤ 2 cm and 1 for diameter > 2 cm; 0 for lymph node-negative and 1 for lymph node-positive disease; and 0 for ER-negative and 1 for ER-positive disease, respectively).

Abbreviations: IGF, insulin-like growth factor; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

*End points for van de Vijver, Wang, and Miller data sets were metastasis-free survival, distant-metastasis-free survival, and disease-specific survival, respectively.

†Tumor grade was binned as 1, 2, and 3 for low, medium, and high, respectively.

‡ER mRNA, PR mRNA, HER2 mRNA, and the IGF gene signature coefficient variables each were transformed to standard deviations from the mean.

be important in the progression to loss of ER and hormone independence. The IGF-I signature was significantly associated with many poor prognostic markers in breast cancer (eg, negative ER/PR status; increasing grade, tumor size, and nodal involvement) and was highly prognostic when both ER-positive and ER-negative tumors were considered together and ER-positive tumors were considered alone (although the signature was not prognostic in ER-negative tumors; results not shown).

Although our data indicate that IGF-I-regulated genes are coordinately expressed in breast cancer and are associated with poor outcome, it is important to note that, because of the inherent cross-talk between numerous growth factor and hormone signaling pathways, the IGF gene signature may in part highlight tumors that are stimulated by hormones or growth factors that share some of the same downstream transcriptional targets. For example, the IGF signature encompasses transcriptional programs downstream of activated Erk1/2 and PI3K/Akt/mTOR. In addition, we found significant overlap between our IGF gene signature and gene signatures derived from MCF-7 cells stimulated with E2 or those that overexpressed EGFR or ERBB2. The overlap in transcriptional programs presumably represents not only the ability of IGFs, E2, and ERBBs to activate similar downstream signaling intermediates (eg, PI3K/Akt) but also the ability of IGFs to directly phosphorylate and activate both ER¹⁹ and ERBBs.²⁰ Although significant overlap was noted with many transcriptional programs, the overlap was often minor (especially in the case of EGFR/ERBB); thus, the IGF signature contains transcriptional programs that are specific and unique to IGF signaling.

We compared our IGF-I signature with other gene signatures previously found prognostic in breast cancer (Appendix Fig A8, online only), including the 70-gene,²¹ wound healing,²² p53-mutation,¹⁶ and HOXB13:IL17BR²³ signatures. We found a small but significant overlap of the IGF-I-induced genes in the wound healing

signature (29 genes overlapped of 233 in the wound healing, activated set), but we found no significant overlap in the 70-gene set. In the van de Vijver breast tumor profile data set, the IGF, 70-gene, p53-mutation, and wound-healing signatures tended to identify many of the same tumors as poor versus good prognosis (Appendix Fig A8A), and such concordance among the prognostic gene signatures has been noted elsewhere.²⁴ Not surprisingly, multivariate Cox analysis (data not shown) indicated that expression of the IGF gene signature was not independent of that of the 70-gene or the wound-healing signature. We found the PI3K gene signature itself (from CMap) to be prognostic and associated with ER negativity in a similar manner to the IGF-I gene signature (results not shown); however, when the PI3K gene signature was removed from the IGF-I signature, the IGF gene signature was still highly prognostic, which indicated that this signature provided information above and beyond PI3K (Appendix Fig A9, online only).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).