

Meta-analysis of Oncogenic Protein Kinase C α Signaling in Lung Adenocarcinoma

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Abstract Purpose: Atypical protein kinase C α (PKC α) is an oncogene in non-small cell lung cancer (NSCLC). Here, we identify four functional gene targets of PKC α in lung adenocarcinoma (LAC), the most prominent form of NSCLC.

Experimental Design: Three independent public domain gene expression data sets were interrogated to identify genes coordinately expressed with PKC α in primary LAC tumors. Results were validated by QPCR in an independent set of primary LAC tumors. RNAi-mediated knockdown of PKC α and the target genes was used to determine whether expression of the identified genes was regulated by PKC α , and whether these target genes play a role in anchorage-independent growth and invasion of LAC cells.

Results: Meta-analysis identified seven genes whose expression correlated with PKC α in primary LAC. Subsequent QPCR analysis confirmed coordinate overexpression of four genes (*COPB2*, *ELF3*, *RFC4*, and *PLS1*) in an independent set of LAC samples. RNAi-mediated knockdown showed that PKC α regulates expression of all four genes in LAC cells, and that the four PKC α target genes play an important role in the anchorage-independent growth and invasion of LAC cells. Meta-analysis of gene expression data sets from lung squamous cell, breast, colon, prostate, and pancreas carcinomas, as well as glioblastoma, revealed that a subset of PKC α target genes, particularly *COPB2* and *RFC4*, correlate with PKC α expression in many tumor types.

Conclusion: Meta-analysis of public gene expression data are useful in identifying novel gene targets of oncogenic PKC α signaling. Our data indicate that both common and cell type-specific signaling mechanisms contribute to PKC α -dependent transformation.

Lung cancer is the leading cause of cancer death in the United States. In 2007, there were an estimated 213,380 new cases and 160,390 deaths from lung cancer (1). The vast majority (~90%) of lung cancer is related to tobacco use (2), the predominant risk factor for this disease. Over the past 30 years, the 5-year survival rate of lung cancer patients has only increased from 7% to 15%, indicating a dire need for more effective means of prevention, diagnosis, prognosis, and treatment. Approximately 80% of lung cancer is classified as NSCLC, which is subclassified into squamous cell carcinoma, lung adenocarcinoma (LAC), and large cell carcinoma. LAC is the most prevalent subclass of lung cancer, accounting for ~50% of non-small cell lung cancer (NSCLC) cases and >40%

of all lung cancers (3). LACs diagnosed at an early stage are often treated with surgery and radiotherapy, whereas patients with unresectable metastatic disease often receive combination chemotherapy (4). Unfortunately, LACs often become resistant to chemotherapy, relapse, and become fatal, underscoring the need for a better understanding of oncogenic signaling mechanisms underlying LAC and the identification of new therapeutic targets for treatment of this disease.

We recently showed that the atypical protein kinase C isozyme PKC α , is an oncogene in human NSCLC (5). PKC α is overexpressed in the majority of primary NSCLC tumors and PKC α expression is predictive of poor clinical outcome independent of tumor stage (5). The PKC α gene, *PRKCI*, is a target for tumor-specific gene amplification in a significant subset of NSCLC tumors, and gene amplification drives PKC α expression in these tumors. PKC α plays a requisite role in NSCLC cell transformation (5, 6). Genetic disruption of PKC α expression inhibits anchorage-independent growth and invasion of NSCLC cells *in vitro*, and tumorigenicity *in vivo* (6). PKC α is a critical downstream effector of oncogenic K-ras, the major oncogenic lesion in NSCLC (5–7).

Given its importance in oncogenic PKC α signaling in lung cancer, we initiated a study to identify downstream targets of PKC α that play a functional role in NSCLC transformation. In the present study, we used a meta-analysis of gene expression data of primary LACs to identify genes whose expression correlates with that of PKC α . Our data show the utility of this approach to identify novel genes involved in oncogenic PKC α

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Translational Relevance

We recently identified protein kinase C ι (PKC ι) as a human oncogene, prognostic marker and therapeutic target in human non-small cell lung cancer (NSCLC). In the present study, we identified four novel PKC ι gene targets whose expression correlates with PKC ι expression in primary NSCLC and whose expression is regulated by PKC ι in NSCLC cells. These genes may be of prognostic significance in patients with NSCLC and other tumors in which PKC ι signaling has been implicated. Like PKC ι , these gene targets represent attractive targets for therapeutic intervention in the treatment of NSCLC. Finally, because expression of these genes is regulated by PKC ι in NSCLC tumors, they may be useful as markers of response to PKC ι -targeted therapies that are currently being developed for clinical use.

signaling and NSCLC biology. These genes also represent potential therapeutic targets.

Materials and Methods

Data collection. Gene expression data from three independent published microarray analyses of LAC tumors were obtained from public sources. The three data sets were as follows: the Michigan data set from Beer et. al. (8) included 86 primary LACs, the Harvard data set from Bhattacharjee et. al. (9) included 136 primary LACs, and the Stanford data set from Garber et. al. (10) included 35 primary LACs. The primary data files were downloaded and rank ordered into tertiles based on PKC ι expression. The expression data were categorized into three tertiles: LACs with High, Medium, and Low PKC ι expression. We defined genes as coordinately expressed with PKC ι if their expression was significantly increased in the High PKC ι expression tertile compared with the Low PKC ι expression tertile, based on two criteria: (a) Student's *t* test, $P < 0.05$, and (b) ≥ 1.4 - or < 0.6 -fold difference in mean gene expression levels between the two tertiles. Because three different expression platforms were used for analysis of these samples, we did not attempt to reanalyze the data but rather worked directly from the processed expression data within the public domain.

Cell lines. Human A549, H358, and H1437 LAC cell lines were obtained from American Type Culture Collection and maintained in adherent culture as suggested by the supplier.

Lentiviral RNA interference-mediated gene knockdown. Lentiviral vectors carrying short hairpin RNA interference (RNAi) against human PKC ι , COPB2, ELF3, PLS1, and RFC4 were obtained from Sigma-

Aldrich Mission shRNA library. A nontarget control lentiviral vector containing a short hairpin that does not recognize any human or mouse genes (NT-RNAi) was used as a negative control in all RNAi experiments. shRNA lentiviral particles were generated as described previously (11). A549, H358, and H1437 cells were stably infected with lentiviral vectors containing short hairpin RNAi-targeting gene of interest or NT-RNAi and selected using 5 μ g/mL puromycin as described previously (11). The RNAi target sequences used in this study are available upon request. Immunoblot analysis of stable transfectants for PKC α , PKC ι , PKC ζ , PKC δ , PKC ϵ , and actin were done as described previously (11). Antibodies were from the following sources: Transduction Laboratories [PKC ι (#610176) and PKC δ (#610397)], Santa Cruz [PKC α (#8393) and PKC ϵ (#214)], and Cell Signaling [PKC ζ (#9372) and β -actin (#4967)].

RNA isolation and QPCR. Total RNA was isolated from lung cancer cell lines and primary lung adenocarcinomas using RNAqueous (Ambion). PKC ι , COPB2, ELF3, PLS1, and RFC4 gene expression assays were purchased from Applied Biosystems to measure mRNA abundance. Analysis was done on an Applied Biosystems 7900 thermal cycler, and data were evaluated using the RQ Manager 1.2 software as described previously (12).

Soft agar growth assays. Anchorage-independent growth was evaluated by the ability of A549 and H358 cell transfectants to form colonies in soft agar as described previously (6). After 4 wk in culture, cells were fixed in 100% methanol and stained with Giemsa. Soft agar colonies were photographed using El Logic100 Imaging System (Eastman Kodak Co.) and quantified using Image Pro Plus 5.0 (Media Cybernetics).

Cellular invasion assay. Cell invasion was assayed using Matrigel-coated Transwell cell culture chambers (Becton Dickinson) as described previously (13). After staining, invasive cells were photographed using an Olympus microscope and quantified using Image Pro Plus 5.0 (Media Cybernetics).

Statistical analysis. The Student's *t* test was used to evaluate the statistical significance of the means of two groups. Kendall Rank Correlation was used to evaluate the strength of the association between two variables. SigmaStat software was used for statistical analyses, and *P* values of < 0.05 were considered statistically significant.

Analysis of coordinate gene expression in other tumor types. Microarray expression data for breast, colon, and prostate cancers were downloaded from the Gene Expression Omnibus (GSE2109), including all 47 prostate adenocarcinoma samples, a randomly selected 82 sample subset of the 195 colon adenocarcinoma samples, and a randomly selected 112 sample subset of the 248 breast ductal carcinoma samples. Microarray expression data for 27 pancreatic carcinomas were downloaded from Array Express (E-MEXP-1121), and microarray expression data for 44 glioblastoma samples were downloaded from The Cancer Genome Atlas (archive files broad.mit.edu_GBU.HT_HG-U133A.1.2.0, broad.mit.edu_GBU.HT_HG-U133A.2.1.0, and broad.mit.edu_GBU.HT_HG-U133A.3.1.0). Raw cel files were processed independently for each cancer type using RMA background correction, fastlo normalization, affinities-only PM correction, and median polish for

Table 1. Identification of genes coordinately expressed with PKC ι in LACs

| Genes | Michigan | | Stanford | | Harvard | |
|----------------|----------|--------------------|----------|---------------------|----------|---------------------|
| | High/Low | <i>P</i> | High/Low | <i>P</i> | High/Low | <i>P</i> |
| <i>PRKCI</i> | 3.41 | 2×10^{-9} | 5.48 | 4×10^{-11} | 2.84 | 5×10^{-14} |
| <i>COPB2</i> | 1.40 | 0.006 | 1.99 | 0.002 | 1.42 | 2×10^{-4} |
| <i>ELF3</i> | 1.67 | 8×10^{-4} | 3.24 | 6×10^{-7} | 1.42 | 0.010 |
| <i>PLS1</i> | 1.92 | 0.007 | 2.47 | 1×10^{-4} | 1.41 | 0.008 |
| <i>RFC4</i> | 1.49 | 0.037 | 4.02 | 9×10^{-7} | 1.44 | 0.007 |
| <i>ALCAM</i> | 1.97 | 0.016 | 1.95 | 0.024 | 1.40 | 0.023 |
| <i>EVPL</i> | 1.56 | 0.012 | 1.78 | 0.009 | 1.80 | 0.004 |
| <i>TACSTD2</i> | 1.52 | 0.008 | 6.71 | 2×10^{-7} | 1.50 | 0.003 |

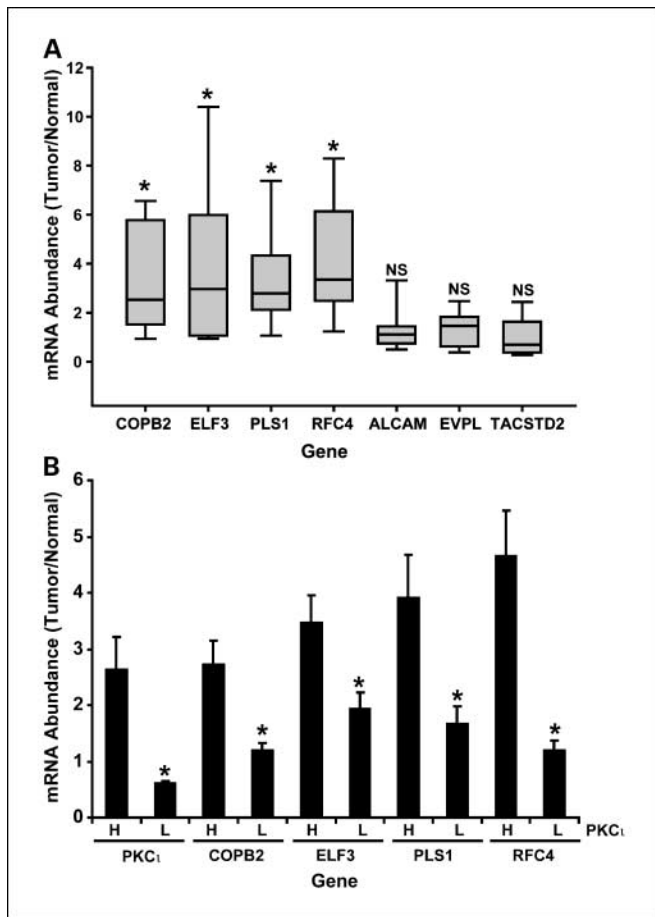


Fig. 1. Identification of genes coordinately expressed with PKC ζ in LAC. *A*, expression of seven genes exhibiting coordinate expression with PKC ζ in primary LAC tumors. Seven genes identified through meta-analysis of public domain gene expression data from LAC cases (see Table 1) were analyzed for overexpression in 15 primary LAC tumors. Four of the seven genes are significantly overexpressed in LACs when compared with matched normal controls. mRNA abundance was measured by QPCR and expressed as fold-change (tumor/normal). Boxes, upper and lower quartile values; line within each box, median value. *, significant difference between tumor and normal with a *P* value of <0.05. NS, not significant. *B*, the abundance of each of the indicated genes was determined by QPCR in 60 primary LAC and matched control samples and expressed as fold change (tumor/normal). The 60 samples were rank ordered and binned into upper tertile (H, high PKC ζ) and lower tertile (L, low PKC ζ) based on PKC ζ expression. The data represent the expression of each gene in the high and low PKC ζ groups. Columns, mean; bars, SE. *, statistically significant difference in expression of the indicated gene in the low PKC ζ group when compared with the high PKC ζ group; *P* < 0.05.

summarization. For each tumor type, the expression data were rank-ordered based on PKC ζ expression, and High, Medium, and Low sample tertiles were identified. Expression levels of the four target genes (COPB2, ELF3, RFC4, and PLS1) in the PKC ζ High tertile were compared with the expression in the PKC ζ Low tertile. Significant changes in expression were measured using a two-tailed, unequal variance, *t* test (*P* < 0.05). The *t* test was computed using the Statistics:TTest PERL library.

Results and Discussion

We recently showed that PKC ζ is an oncogene in NSCLC (5). To identify potential downstream targets of PKC ζ , we performed a meta-analysis of public domain gene expression data of primary human LACs. Three independent gene

expression data sets (hereafter called the Michigan, Stanford, and Harvard data sets, respectively) were downloaded from public sources as described in Materials and Methods. Our strategy was to interrogate these data sets for genes whose

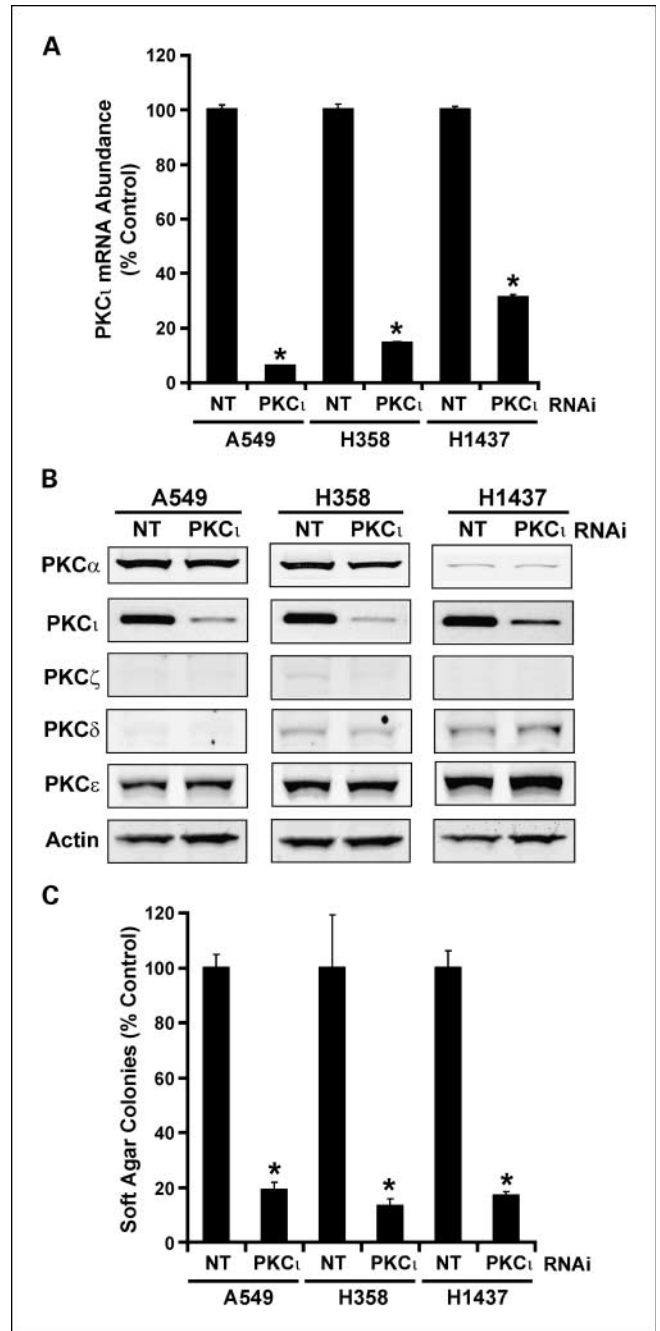


Fig. 2. RNAi-mediated knockdown of PKC ζ inhibits anchorage-independent growth of LAC cell lines in soft agar. A549, H358, and H1437 LAC cell lines were stably transfected with either a nontarget (NT) or lentiviral RNAi construct of a PKC ζ -RNAi construct. *A*, expression of PKC ζ mRNA in NT and PKC ζ -RNAi cells. *, statistically significant decrease in PKC ζ mRNA abundance when compared with NT control. Data are expressed as % NT control; *n* = 3; *P* < 0.05. *B*, PKC ζ -RNAi leads to selective knockdown of PKC ζ protein expression. Immunoblot analysis of total cell lysates from A549, H358, and H1437 cells treated with NT or PKC ζ -RNAi for PKC α , PKC ζ , PKC δ , PKC ϵ , and actin. *C*, RNAi-mediated knockdown of PKC ζ inhibits anchorage-independent growth of LAC cell lines. Anchorage-independent growth in soft agar was assessed as described previously (6). Columns, mean (*n* = 5); bars, SE. Data are expressed as % NT control. *, statistically significant difference from NT control; *P* < 0.05.

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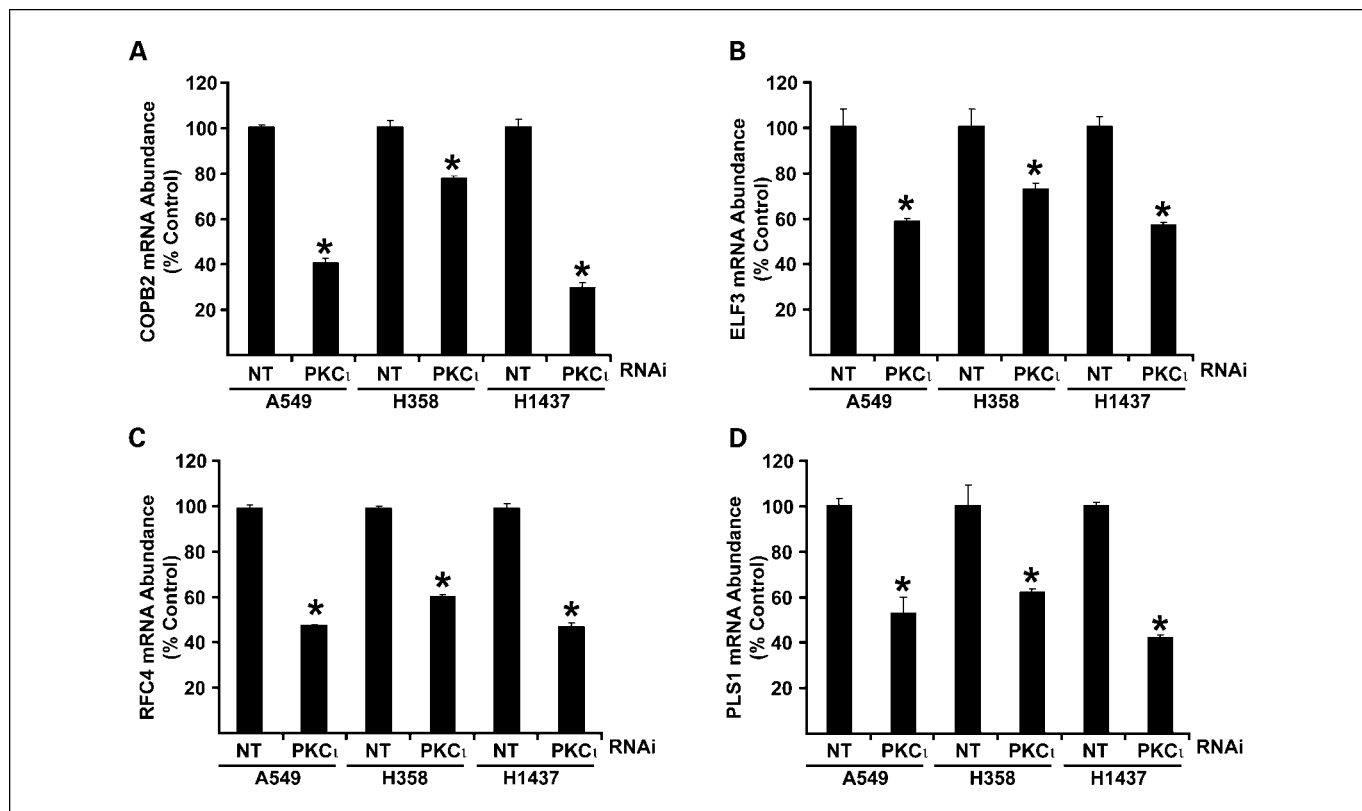


Fig. 3. PKC ι regulates the expression of COPB2, ELF3, PLS1, and RFC4 in LAC cells. A549, H358, and H1437 cells transfected with either NT or PKC ι -RNAi were analyzed for expression of the four genes that are coordinately overexpressed with PKC ι in LAC tumors. RNAi-mediated knockdown of PKC ι causes a statistically significant decrease in the expression of COPB2 (A), ELF3 (B), RFC4 (C), and PLS1 (D) in all three LAC cell lines. Data are expressed as %NT control; columns, mean ($n = 4$); bars, SE. *, statistically significant difference when compared with the corresponding NT control. $P < 0.05$.

expression is coordinately regulated with PKC ι in LAC. For this purpose, we force ranked the samples from each data set based on PKC ι expression, binned the samples into tertiles based on PKC ι expression, and interrogated the data sets for genes whose expression correlated with PKC ι (either negatively or positively). This process was repeated for all three data sets, and only genes that correlated with PKC ι in all three data sets were considered further. Seven genes were identified that satisfied the inclusion criteria, *COPB2*, *ELF3*, *PLS1*, *RFC4*, *ALCAM*, *EVPL*, and *TACSTD2*. Table 1 summarizes the results of this analysis, including the fold-change and t test P values obtained from comparing the expression of each of these genes in the high (H) and low (L) sample set tertiles. Each gene showed a positive correlation with PKC ι expression, and no genes were identified from this analysis whose expression was negatively correlated with PKC ι .

Because we were interested in identifying genes that might be functionally important in oncogenic PKC ι signaling, we chose to focus our attention on genes whose expression, like that of PKC ι , was induced in LAC tumors when compared with matched normal lung tissue. For this purpose, we measured the expression of each of the 7 genes identified above in a test set of 15 primary LAC samples for which matched normal RNA was available (Fig. 1A). Four of the seven genes identified in our original analysis were significantly overexpressed in LAC tumors when compared with matched normal tissues (*COPB2*, *ELF3*, *PLS1*, and *RFC4*). Expression of *ALCAM*, *EVPL*, and

TACSTD2 were not significantly elevated in LAC tumors when compared with matched normal lung tissue and therefore were not analyzed further. We further validated the coordinate overexpression of the 4 identified genes with PKC ι in an expanded set of 60 primary LAC samples and corresponding matched normal lung tissues. PKC ι expression was measured by QPCR, samples binned into tertiles based on PKC ι expression, and expression levels of the four target genes determined by QPCR. The data were then evaluated for an association between PKC ι expression and that of each of the four target genes (Fig. 1B). Our analysis revealed that expression of each of the four genes exhibited a statistically significant association with PKC ι expression. Thus, four of the seven genes originally identified as being coordinately expressed with PKC ι in LAC were subsequently confirmed by QPCR in an independent set of LAC tumors.

One purpose of our analysis was to identify potential downstream effectors of oncogenic PKC ι signaling in LAC. Therefore, we next assessed whether the expression of any of the four PKC ι -associated genes was regulated by PKC ι in LAC cell lines. For this purpose, we generated three LAC cell lines in which PKC ι expression was knocked down by RNAi using RNAi reagents and methods described and characterized previously (11). RNAi-mediated knockdown of PKC ι caused significant inhibition of PKC ι expression in each of the three LAC cell lines (Fig. 2A). Immunoblot analysis of lysates from A549, H358, and H1437 cells treated with NT and PKC ι RNAi revealed a

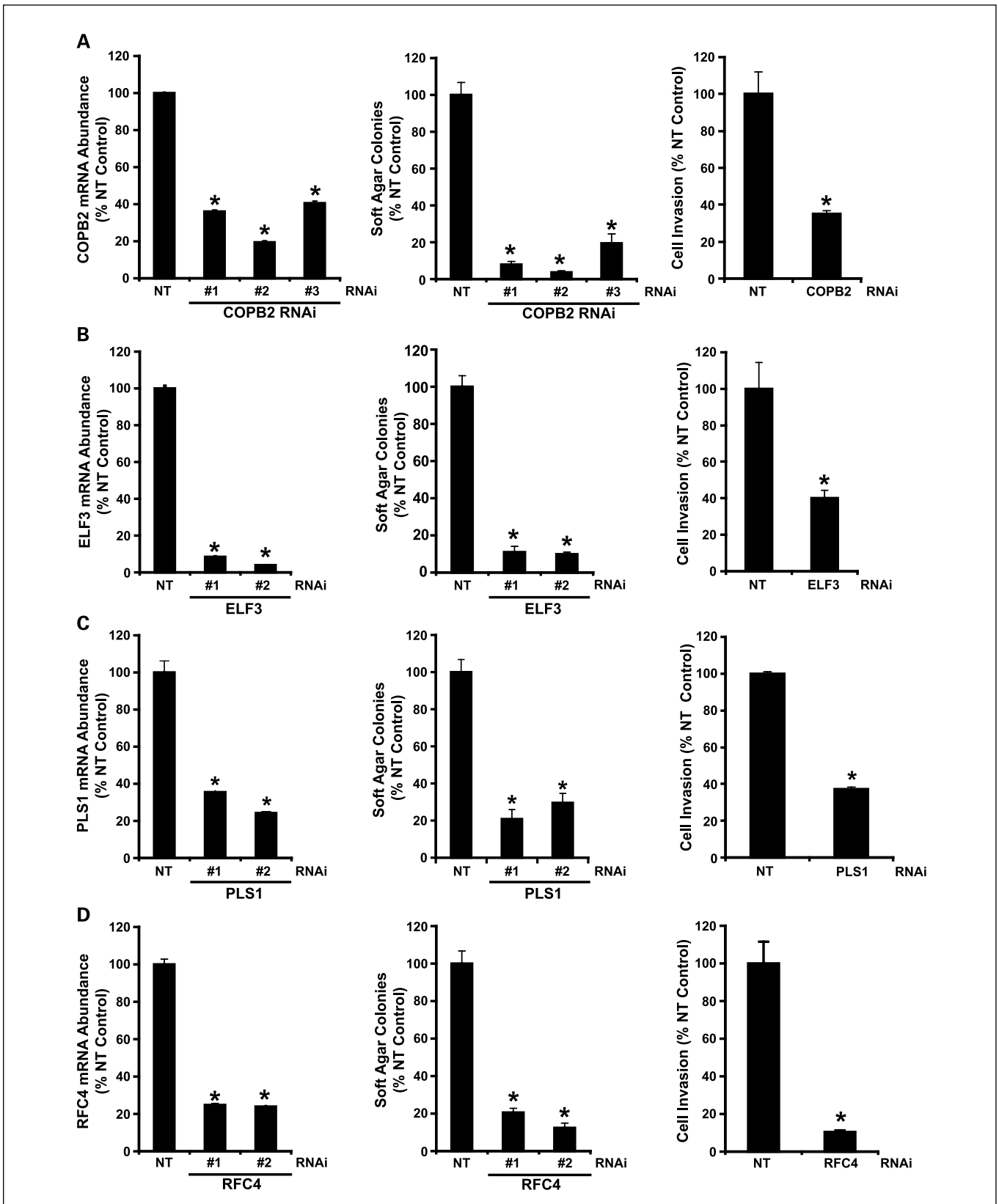


Fig. 4. COPB2, ELF3, PLS1, and RFC4 are functionally important for LAC cell transformation. A549 cells were stably transfected with multiple independent RNAi constructs targeting COPB2 (A), ELF3 (B), PLS1 (C), RFC4 (D), or a NT control RNAi construct. Target gene mRNA abundance was determined by QPCR in A549 cells expressing NT or target gene RNAi. Columns, mean ($n = 3$) expressed as % NT control; bars, SE. *, statistically significant difference when compared with NT control cells. $P < 0.05$. The effect of target RNAi on anchorage-independent growth in soft agar and cellular invasion through Matrigel-coated chambers was assessed as previously described (13). Data are presented as % NT control; columns, mean ($n = 4$); bars, SE. *, statistically significant difference from NT control; $P < 0.05$.

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significant decrease in expression of PKC ι protein but no demonstrable change in expression of PKC α , PKC ζ , PKC δ , or PKC ϵ (Fig. 2B), demonstrating specific knockdown of PKC ι expression by our PKC ι RNAi construct. We previously showed that PKC ι is required for anchorage-independent growth of NSCLC cell lines *in vitro* and tumorigenicity *in vivo* (5, 6). Therefore, we assessed the effect of PKC ι knockdown on anchorage-independent growth in soft agar (Fig. 2C). As expected, each of the three LAC cell lines in which PKC ι was knocked down exhibited a significant inhibition of anchorage-independent growth when compared with nontarget control cells. Having shown effective knockdown of PKC ι and consequent inhibition of PKC ι -dependent transformed growth, we next assessed the effect of PKC ι knockdown on the expression of the four target genes. As seen in Fig. 3, PKC ι knockdown led to a significant inhibition in expression of each of the four target genes *COPB2* (Fig. 3A), *ELF3* (Fig. 3B), *PLS1* (Fig. 3C), and *RFC4* (Fig. 3D) in all three LAC cell lines. Our data indicate that PKC ι regulates the expression of each of the four target genes in multiple LAC cell lines.

We next assessed whether the four PKC ι target genes play a functional role in LAC cell transformation (Fig. 4). For this purpose, we established lentiviral RNAi reagents that target each gene. A549 cells were stably transfected with three lentiviral RNAi constructs directed against *COPB2*, and stably transfected cell populations were analyzed by QPCR for expression of *COPB2* mRNA (Fig. 4A). Each of the RNAi constructs significantly inhibited *COPB2* mRNA abundance when compared with a nontarget RNAi control. A549/*COPB2* RNAi cells were assessed for their ability to grow as colonies in soft agar (Fig. 4A). Each of the *COPB2* RNAi transfectants exhibited a significant inhibition of anchorage-independent growth in soft agar when compared with nontarget control cells. The inhibition of soft agar growth was commensurate with the level of inhibition of *COPB2* expression. A549 cells expressing the most effective *COPB2* RNAi construct (*COPB2* #2) were also assessed for ability to invade through Matrigel-coated chambers (Fig. 4A). A significant decrease in cellular invasion was observed in *COPB2* knockdown cells when compared with NT control cells. We conclude that *COPB2* plays a significant role in the transformed phenotype of A549 LAC cells.

A similar analysis involving RNAi-mediated knockdown of the three other PKC ι regulated genes, *ELF3* (Fig. 4B), *PLS1*

(Fig. 4C), and *RFC4* (Fig. 4D), was also conducted. In each case, multiple RNAi constructs targeting the gene of interest induced significant inhibition of target gene expression and a commensurate decrease in anchorage-independent growth and cellular invasion of A549 cells through Matrigel. Taken together, these data show that each of these PKC ι -regulated genes plays a functional role in NSCLC cell transformation. Similar results were obtained in H358 cells (data not shown), indicating that the observed effects are not specific to A549 cells.

Having shown the utility of using meta-analysis of gene expression data to identify relevant targets of oncogenic PKC ι in LAC, we next wished to assess whether these genes are also PKC ι targets in other major tumor types. Therefore, we obtained gene expression data from the public domain for lung squamous cell carcinoma, carcinomas of the breast, colon, prostate, and pancreatic, as well as glioblastoma, and analyzed these data sets for coordinate expression of the four PKC ι -regulated genes with PKC ι (Table 2). Of the four genes identified in LAC, *COPB2* and *RFC4* showed statistically significant coordinate expression with PKC ι in several other tumor types. *COPB2* showed highly significant coordinate expression with PKC ι in all major tumor types tested, and *RFC4* showed coordinate expression in a majority of tumor types. *ELF3* showed coordinate overexpression with PKC ι in LAC and glioblastoma, whereas *PLS1* exhibited coordinate expression with PKC ι only in LAC. Taken together, these data indicate that PKC ι regulates oncogenic signaling through both common and tumor type-specific mechanisms.

Our analysis identified four genes whose expression is regulated in human tumors by oncogenic PKC ι —*COPB2*, *RFC4*, *PLS1*, and *ELF3*. Each of these genes is involved in human LAC cell transformation. Our data suggest possible key signaling pathways that may contribute to PKC ι -dependent transformation. *COPB2* is a subunit of the coatamer, a cytoplasmic protein complex that binds dilysine motifs and associates with Golgi nonclathrin-coated vesicles (14). The coatamer functions to mediate biosynthetic protein transport from the endoplasmic reticulum via the Golgi up to the *trans*-Golgi network. The coatamer is also required for Golgi membrane budding and is essential for the retrograde Golgi to endoplasmic reticulum transport of proteins. The coatamer is also involved in membrane trafficking in the exocytic pathway and for endocytic recycling of cell surface receptors. The association of the coatamer with the Golgi is regulated by binding of ADP ribosylation factors and is GTP dependent. Interestingly, *COPB2* was previously identified as a binding partner for the PKC ϵ (15). Whether *COPB2* also binds PKC ι remains to be determined. These data suggest that PKC ι may regulate Golgi function and membrane trafficking in tumor cells. Our data are the first to our knowledge to directly implicate *COPB2* in transformation.

RFC4 is a clamp loader protein that binds in a complex with proliferating cell nuclear antigen to regulate elongation of primed DNA templates by DNA polymerases (16, 17). The clamp loader also functions in unscheduled DNA repair and check point control through interactions with the RAD17 911 DNA damage checkpoint complex (18). The fact that PKC ι regulates *RFC4* expression suggests that PKC ι may function to modulate either DNA synthesis and/or DNA damage check-point function in tumor cells. *ELF3* is a transcriptional factor in the ETS domain family and is known to regulate expression of

Table 2. Coordinate expression of PKC ι target genes in major cancer types

| Tumor type (number) | <i>COPB2</i> | <i>RFC4</i> | <i>ELF3</i> | <i>PLS1</i> |
|--------------------------|--------------------|--------------------|-------------|-------------|
| Lung squamous cell (129) | 2×10^{-6} | 2×10^{-8} | NS | NS |
| Breast (112) | 0.016 | 0.003 | NS | NS |
| Colon (82) | 0.008 | NS | NS | NS |
| Prostate (47) | 0.02 | NS | NS | NS |
| Pancreas (27) | 0.05 | NS | NS | NS |
| Glioblastoma (44) | 0.016 | NS | 0.04 | NS |

NOTE: *P* values are listed for the correlation between expression of PKC ι and the indicated target gene.
Abbreviation: NS, not significant.

many growth-related genes including angiopoietin 1, collagenase, and other transformed growth and invasion-related genes (19, 20). Interestingly, ELF3 expression has been shown to be largely restricted to epithelial cells of the lung, intestinal tract, and kidney. ELF3 has also been shown to be highly expressed in lung carcinoma tissues and in lung cancer cell lines when compared with normal lung tissue, suggesting a specific role for ELF3 in the lung (20). Our data indicate that ELF3 expression in lung tumors is regulated by oncogenic PKC ζ and that ELF3 plays a critical role in lung tumorigenesis. Our finding that ELF3 expression does not correlate with PKC ζ expression in other tumor types is consistent with the restricted expression of ELF3 to the lung observed by others. Finally, PLS1 encodes plastin, an actin-, calcium-binding protein involved in the regulation of the actin cytoskeleton (21). Interestingly, plastin has previously been reported to be overexpressed in prostate cancer cells and that genetic disruption of plastin expression with antisense mRNA inhibits invasion of prostate cancer cells (22, 23). Our data confirm the importance of plastin in tumor cell invasion and identify it as a key target of PKC ζ -mediated NSCLC tumor invasion. This report is the first to provide direct evidence for the involvement of most of these genes in the transformed phenotype of human tumor cells. Because so little

is known about these proteins in the context of transformation, future studies will be required to elucidate the mechanisms by which these genes impinge on anchorage-independent growth and invasion. In conclusion, our results show the utility of using meta-analysis of public domain gene expression data sets to identify novel genes involved in cellular transformation downstream of a newly identified oncogene, PKC ζ . Because expression of these genes has been documented in primary human tumors, these genes, and the pathways in which they operate are attractive targets for development of novel prognostic markers and biomarkers, and in new therapeutic strategies for treatment of cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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