Gene regulation by phorbol 12-myristate 13-acetate in MCF-7 and MDA-MB-231, two breast cancer cell lines exhibiting highly different phenotypes

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Abstract. We have examined the effects of the protein kinase C (PKC)-activator phorbol 12-myristate 13-acetate (PMA) on gene expression in two breast cancer cell (BCC) lines exhibiting highly different phenotypes. These are the estrogen receptor α (ERα)-positive, weakly invasive, luminal epithelial-like MCF-7 and the ERα-negative, highly invasive, fibroblast-like MDA-MB-231. They express constitutively low and high PKC activities, respectively. After a 24-h exposition to 100 nM PMA, the number of genes showing an altered expression at the 2-fold change level was much higher in MCF-7 (n=435) than in MDA-MB-231 (n=18) BCC. Four of these genes, namely *CDC2, CENPA, NR4A1* and *MMP10*, were altered in the same way in both cell lines. Two genes were regulated in an opposite way: *ID1* and *EVA1*. Many of the genes down-regulated in MCF-7 BCC appeared to be preferentially expressed in the G_1, S, and/or G_2 phases of the cell cycle. The ERα gene, *ESR1*, and other genes associated to the ERα-positive, luminal epithelial-like BCC phenotype were down-regulated, while a series of genes related to a more aggressive, fibroblast-like BCC phenotype were up-regulated. Other altered genes were notably linked to cell architecture, supporting profound effects of PMA on cell morphology and motility, as well as on the interactions between BCC and their neighboring proteins. Of note, all the modulated genes involved in proteolysis and its control were up-regulated. In summary, PMA effects suggest that PKC activation may induce, to some extent, a more fibroblast-like phenotype in the ERα-positive, luminal epithelial-like MCF-7 BCC, and significantly modulate the interactions of these cells with their environment.

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Introduction

Based on the initial observation that it was able to promote skin tumor formation, phorbol 12-myristate 13-acetate (PMA) has been widely used in experimental oncology. PMA is an activator of various isoforms of protein kinase C (PKC). Total PKC activity is elevated in breast cancer tissues, suggesting an association with tumorigenicity (1). In breast cancer cell (BCC) lines, a relation has been found between a high PKC activity and the absence of estrogen receptor α (ERα), and PKC activity is generally higher in aggressive and invasive BCC lines (2). Treatment with PMA has been shown to drastically increase the invasiveness of low-PKC-expressing, ERα-positive MCF-7 BCC. A PKC inhibitor (H7) reversed the PMA effects on these cells. Similar effects of PMA were observed for cell chemo-taxis (3). A prolonged (hours) exposure of MCF-7 to PMA led to a significant growth reduction, an effect seen to a much lesser extent in MDA-MB-231 cells (4). In MCF-7 BCC stably transfected with the PMA-responsive protein kinase C α isoform, various changes were observed: enhanced proliferation rate, anchorage-independent growth, dramatic morphologic alterations including loss of an 'epithelioid' appearance, significant increase in the level of the mesenchymal/fibroblastic marker vimentin, increased tumorigenicity in nude mice, a significant reduction in ERα expression, and a decrease in estrogen-dependent gene expression (5). These observations suggest that PKC activation by PMA may produce considerable changes in gene expression, at least in MCF-7 BCC.

The introduction of DNA micro-array use in oncology may notably allow dissecting the gene response associated to the activation of specific signaling pathways. Here, we have applied this technology to examine the gene expression patterns in MCF-7 and MDA-MB-231 cells exposed to PMA.

Materials and methods

Cell culture. MCF-7 and MDA-MB-231 cells were obtained from ATCC (Rockville, MD, USA). These BCC lines express very different phenotypes. MCF-7 cells are ERα-positive, weakly invasive *in vitro*, and exhibit a series of markers characteristic of the luminal cells of the breast epithelium.

Table I. The 50 genes most up- or down-regulated by PMA in MCF-7 BCC.

Description	Gene name	GB Acc	AVG
Up-regulation			
Matrix metalloproteinase 1 (interstitial collagenase)	MMP1	NM_002421	85.40
Protein kinase, cAMP-dependent, catalytic, α	PRKACA	NM_002730	76.28
Prostate differentiation factor	PLAB	AU123196	47.89
S100 calcium binding protein A9 (calgranulin B)	S100A9	BG331778	38.95
S100 calcium binding protein P	S100P	AI148603	26.73
Serine (or cysteine) proteinase inhibitor, clade A, member 3	SERPINA3	BF339409	24.08
Aldo-keto reductase family 1, member C4	AKR1C4	AV694764	20.88
Leratin, hair, basic, 1	KRTHB1	BE785699	18.82
Matrix metalloproteinase 10 (stromelysin 2)	MMP10	NM_002425	18.76
Baculoviral IAP repeat-containing 3	BIRC3	AI417860	17.84
Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A	AA481712	15.82
Glutaredoxin (thioltransferase)	GLRX	BG399074	13.29
Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CDKN1A	L26165	12.55
Orosomucoid 1	ORM1	XM_011748	11.59
Aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1)	AKR1C1	BE786113	11.51
Matrix metalloproteinase 10 (stromelysin 2)	MMP10	BE740153	9.68
Potassium voltage-gated channel, subfamily F, member 1	KCNF1	NM_002236	7.19
Protein kinase H11	H11	NM_014365	7.10
Lymphotoxin beta (TNF superfamily, member 3)	LTB	AW188005	6.94
Retinoic acid induced 3	RAI3	BF212425	6.21
Keratin, hair, basic, 3	KRTHB3	NM_002282	5.87
Elongation factor, RNA polymerase II, 2	ELL2	NM_012081	5.82
Diacylglycerol O-acyltransferase homolog 2 (mouse)	DGAT2	AW188221	5.62
Matrix metalloproteinase 9	MMP9	NM_004994	4.91
Protein kinase H11	H11	NM_014365	4.20
Down-regulation			
T cell receptor gamma locus	TRGV9	AI972955	0.08
Insulin-like growth factor binding protein 5	IGFBP5	AA374325	0.13
Exonuclease 1	EXO1	AC004783	0.14
S-phase kinase-associated protein 2 (p45)	SKP2	BF311153	0.14
Adducin 3, γ	ADD3	NT_030059	0.15
Nuclear receptor subfamily 2, group F, member 2	NR2F2	NM_021005	0.16
Adducin 3, γ	ADD3	AL135243	0.18
Proliferating cell nuclear antigen	PCNA	NM_002592	0.18
EST	EST	H02833	0.18
v-myb myeloblastosis viral oncogene homolog (avian)	MYB	BG387620	0.19
Thioredoxin interacting protein	TXNIP	AU139227	0.19
Butyrobetaine (γ), 2-oxoglutarate dioxygenase 1	BBOX1	R41886	0.20
KIAA1441 protein	KIAA1441	AL519335	0.20
Sperm specific antigen 2	SSFA2	XM_002507	0.20
KIAA0101 gene product	KIAA0101	AV716856	0.20
Epithelial protein lost in neoplasm ß	EPLIN	AK023649	0.21
Cyclin E2	CCNE2	NM_004702	0.21
Butyrylcholinesterase	BCHE	AU117425	0.21
KIAA0534 protein	KIAA0534	AB011106	0.21
Insulin-like growth factor binding protein 5	IGFBP5	AU132011	0.21
Replication factor C (activator 1), 4	RFC4	AF538718	0.21
Butyrylcholinesterase	BCHE	NM_004056	0.21
Thymidylate synthetase	TYMS	AI174883	0.21
vav 3 oncogene	VAV3	XM_010540	0.22
MCM2 minichromosome maintenance deficient 2, mitotin (*S. cerevisiae*)	MCM2	BE250461	0.22

MDA-MB-231 BCC are ERα-negative, highly invasive *in vitro*, and share a series of markers with mesenchymal cells such as fibroblasts. However, their epithelial nature is notably supported by the fact that they express, although to a low extent, the cytokeratins 8, 18, and 19 (6) (for an extensive review on BCC lines, including MCF-7 and MDA-MB-231, see ref. 7). Both cell lines were routinely cultured in MEM medium added with 5% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (Life Technologies). PMA was from Sigma.

RNA extraction and probe preparation. Isolation of RNA was performed using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The quality of the RNA was assessed based on the RNA profile generated by the bioanalyzer (Agilent Inc.). Total RNA was linearly amplified using a modification of the Eberwine method (8). Briefly, total RNA was reverse-transcribed by using a 63 nucleotide synthetic primer containing the T7 RNA polymerase binding site (5'-GGCCAGTGAATTGTAATACGACTCACTATA GGGAGGCGG$(T)_{24}$-3'. Second strand cDNA synthesis (producing double-stranded cDNA) was performed with RNase H, *E. coli* DNA polymerase I, and *E. coli* DNA ligase (Invitrogen, Carlsbad, CA). After cDNA was blunt-ended with T4 DNA polymerase (Invitrogen), it was purified by phenol-chloroform-isoamyl alcohol extraction and ammonium acetate-ethanol precipitation. The double-stranded cDNA was then transcribed using T7 polymerase (T7 Megascript Kit, Ambion, Austin, TX), yielding amplified anti-sense RNA which was purified using RNeasy mini-columns (Qiagen, Valencia, CA). Total RNA from the Universal Human Reference (UHR) (Stratagene) was amplified and used as reference for cDNA micro-array analysis. The cDNA micro-array chips consisted of 10,369 total features, and were manufactured at the Jules Bordet Institute micro-array facility.

Amplified RNA (3 μg) was reverse-transcribed and directly labeled using cyanine 5-conjugated dUTP (tumor RNA) or cyanine 3-conjugated dUTP (UHR). Hybridization was performed in the presence of 5X SSC and 25% formamide for 14-16 h at 42˚C. Slides were washed, dried, and scanned using an Axon 4000a laser scanner. A detailed protocol for RNA amplification as well as cDNA probe labeling and hybridization is available on the web at http://nciarray.nci.nih.gov/reference/index.shtml. To exclude labeling biases, amplified RNA from each cell line and condition were labeled with the reciprocal fluorochrome in a duplicate experiment.

Scanning and data processing. Following hybridization, micro-arrays were scanned using a 10 μm resolution GenePix 4000 scanner (Axon Instruments, Inc., Foster City, CA) at variable PMT (photo-multiplier tube) voltage to obtain maximal signal intensity with <1% probe saturation. Resulting TIFF images for each fluorescent were analyzed with GenePix software version 3.0 (Axon Instruments, Inc., Foster City, CA). The data files generated by GenePix v3.0 were entered into a web-based database maintained by the Microarray Unit, Jules Bordet Institute.

Data analysis. Expression profiles were analyzed using BRB Array tools, version 3.0 (Molecular Statistics and Bioinformatics Section, National Cancer Institute, Bethesda, USA). For each fluorescent channel, the log-ratios were normalized by subtracting the median log-ratio from each experiment. Spots of any experiment, which had red and/or green intensity <250 units after subtraction to the background, a spot size <25 pixels or flagged for any experimental reason were filtered out. Differentially expressed genes were designated significant if they were reproducibly induced by >2-fold in the two reciprocal experiments for each cell line and condition.

A Java-based program called Onto-Express (9) was used to determine whether clusters of genes with similar expression profiles were enriched in specific GO functional categories. Based on the genes present on the chip, Onto-Express calculated the expected number of occurrences of each functional category in each cluster. For the current experiments, a binomial model was selected to calculate the probability that each functional category was over-represented in a cluster and the p-values were corrected for the multiplicity using the false discovery rate method (10).

Results and Discussion

Differential gene regulation by PMA in MCF-7 and MDA-MB-231 BCC. Upon a 24-h exposure to 100 nM PMA, the expression of 197 genes was increased and that of 238 genes was decreased >2-fold in MCF-7 BCC. The 25 most up- or down-regulated genes in MCF-7 are listed in Table I. In MDA-MB-231 cells, only 18 genes were modulated (9 down- and 9 up-regulated, as shown in Table II). This considerable difference is in line with previous data indicating that the growth-arrest effect of PMA was much higher in MCF-7 than in MDA-MB-231 cells. This has been attributed to the presence of higher levels of the δ isoform of PKC (PKCδ) in MCF-7 (11). Whether the PKCδ level may also explain the differential modulation of gene expression by PMA in the two cell lines is, however, presently unknown.

We found that PMA notably decreased the PKCδ gene (*PRKCD*) expression level in MCF-7 cells (Table I). Previous observations have related decreased PKCδ amount to the acquisition of a more aggressive neoplastic phenotype in MCF-7 cells stably transfected with the alpha isoform of PKC (PKCα) (5). However, the growth-arrest response of BCC to PMA has been linked to their PKCδ level. We suggest that a PKCα-mediated reduction of PKCδ activity in MCF-7 cells could partly desensitize them to the growth-inhibiting effects of the phorbol ester.

In contrast to PKCδ, constitutive PKCα and total PKC activities are much lower in MCF-7 than in MDA-MB-231 BCC (2,3). PMA treatment may enhance these PKC activities to a much higher extent in the former than in the latter cells, which we expected to result in a more extensive modulation of gene expression in MCF-7 cells. This was effectively observed here. On the other hand, a number of genes that were up- or down-regulated by PMA in MCF-7 are known to be constitutively expressed at a high or low-level, respectively, in MDA-MB-231 cells. This is notably the case for genes associated to the fibroblast-like and luminal epithelial-like phenotypes (see below). We suggest that the amount of expression of these genes could not be further altered by PMA in MDA-MB-231 BCC.

Table II. Genes up- or down-regulated by PMA in MDA-MB-231 BCC.

Description	Gene name	GB Acc	AVG
Up-regulation			
Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 2	SERPINB2	AL550163	9.85
Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	PTGS2	AL033533	6.35
Tissue factor pathway inhibitor 2	TFPI2	AL550357	5.98
Matrix metalloproteinase 10 (stromelysin 2)	MMP10	NM_002425	3.03
Interleukin 1, ß	IL1B	W38319	2.84
Secretory granule, neuroendocrine protein 1 (7B2 protein)	SGNE1	BE047084	2.47
Peroxisome proliferative activated receptor, δ	PPARD	AU131142	2.42
Nuclear receptor subfamily 4, group A, member 1	NR4A1	XM_006843	2.15
Matrix metalloproteinase 3 (stromelysin 1)	MMP3	XM_006271	2.14
Down-regulation			
Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	ID1	AL117381	0.34
Synuclein, α (non A4 component of amyloid precursor)	SNCA	AW156890	0.38
Cyclin A1	CCNA1	NM_003914	0.41
Serine (or cysteine) proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 5	SERPINA5	NM_000624	0.42
Profilin 2	PFN2	AV724105	0.44
Epithelial V-like antigen 1	EVA1	AF275945	0.44
Centromere protein A (17 kDa)	CENPA	AL555786	0.45
Cell division cycle 2	CDC2	NT_008583	0.46
Hephaestin	HEPH	AW960584	0.48

Genes modulated by PMA in MCF-7. By using the Gene Ontology software, genes having an expression level altered by PMA in MCF-7 BCC were classified according to three criteria, based on their protein product. These criteria were: a) the molecular function of the protein (Fig. 1A); b) the biological process in which it is involved (Fig. 1B); c) the cellular component where it is localized (Fig. 1C). Regarding function, a significantly high number of genes were associated to ATP binding, DNA binding, hydrolase activity, receptor activity, and protein serine/threonine kinase activity. This is consistent with energy-driven changes in signalling and DNA-associated events. Gene classification according to biological processes supported an important role for PMA in DNA metabolism and regulation of cell cycle/apoptosis. Finally, the cellular component analysis confirmed the extent of nuclear events in PMA action (Fig. 1).

A literature-based analysis of the modulated genes was also conducted. We found that about 22% of the genes regulated by PMA in MCF-7 cells were known to be expressed at a higher level in late G_1, S, and/or G_2 phases of the cell cycle (see notably ref. 12). The great majority of these genes were down-regulated, in agreement with the well-known growth-arrest effect of PMA (4,11).

The expression of a series of genes associated to the ERα-positive, luminal epithelial-like phenotype of BCC was also decreased. These genes included the ERα gene (*ESR1*) itself, estrogen-induced genes such as *BCL2* (B-cell CLL/lymphoma 2), *LIV-1* [now *SLC39A12* - solute carrier family 39 (zinc transporter), member 12], *PGR* (progesterone receptor), *RERG* (RAS-like, estrogen-regulated, growth-inhibitor), *TFF3* (trefoil factor 3) and other genes known to be co-expressed with the ER in BCC lines, such as *GPR30* (G protein-coupled receptor 30), *IGFBP5* (insulin-like growth factor binding protein 5), *MYB* (v-myb avian myeloblastosis viral oncogene homolog), *NAT1* (N-acetyltransferase 1), *SELENBP1* (selenium-binding protein 1), *SYK* (spleen tyrosine kinase), *VAV3* (vav 3 oncogene) (reviewed in ref. 7). Another down-regulated gene, *AIBZIP* (now *CREB3L4* - cAMP responsive element binding protein 3-like 4), was previously shown to be androgen-inducible and expressed in androgen-dependent but not -independent prostate cell lines (13). Our data suggest that it could be similarly related to the estrogen-responsive status of BCC lines.

In contrast, a series of genes associated to the fibroblast-like and/or ERα-negative phenotype of BCC were up-regulated. Expression of most of these genes has been previously reported to be lower in MCF-7 than in MDA-MB-231 cells. These genes included *AREG* (amphiregulin), *ACTG1* (actin γ1), *CEBPB* [CCAAT/ enhancer binding protein (C/EBP), ß], *CTSL* (cathepsin L), *FN1* (fibronectin), *ICAM1* (intercellular cell adhesion molecule-1), *ITGA3* (integrin α 3 chain), *ITGA5* (integrin α 5 chain), *LAMB3* (laminin ß 3 chain), *LIF* (leukemia inhibitory factor), *MMP1* (matrix metalloproteinase 1), *MMP9* (matrix metalloproteinase 9), *MMP10* (matrix metalloproteinase 10), *NRP1* (neuropilin 1), *VEGF* (vascular endothelial growth factor).

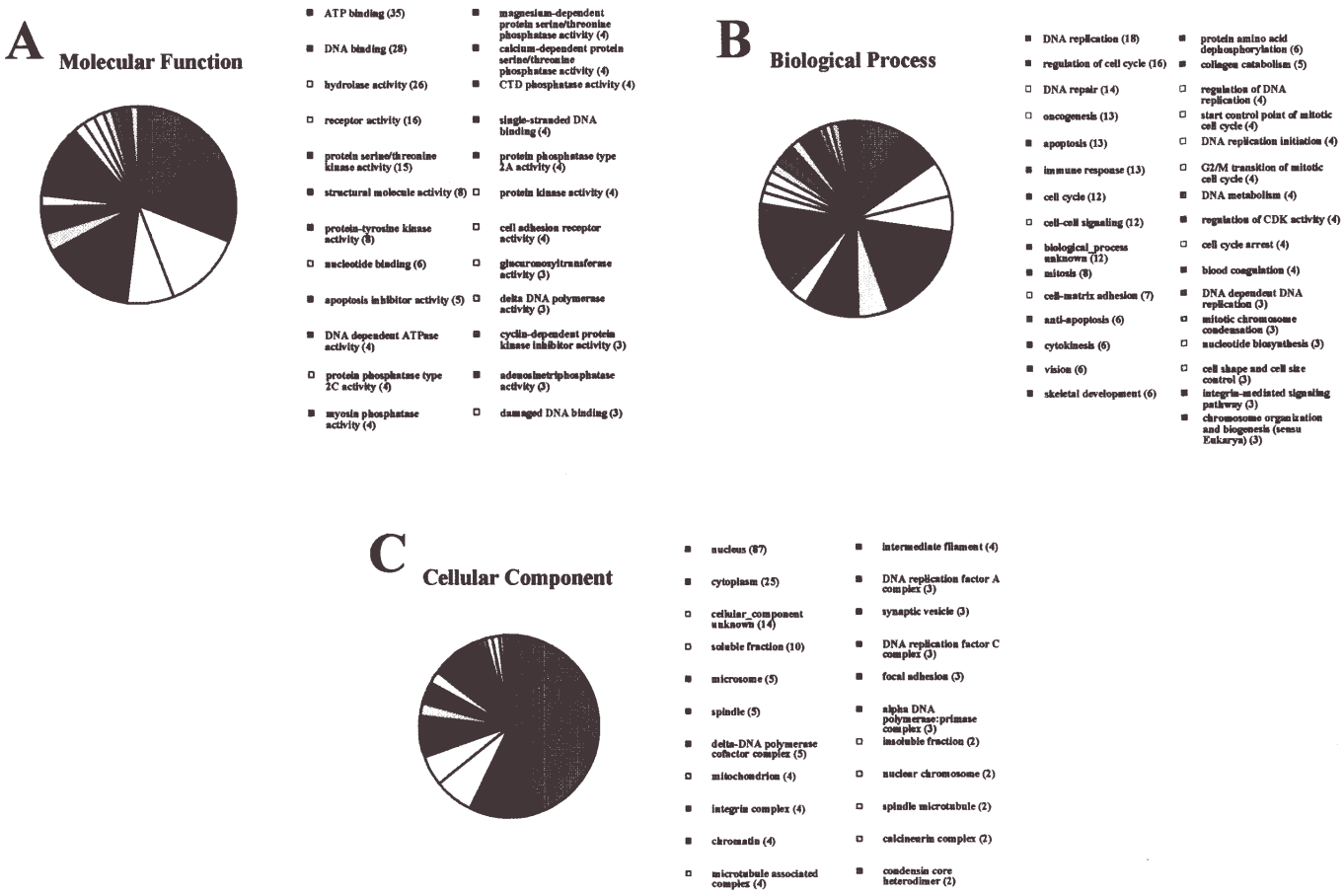

Figure 1. Gene Ontology (GO) analysis data. Distribution of genes modulated by PMA in MCF-7 BCC according to the molecular function (A), biological processes (B) and cellular components (C).

The data indicating that PMA may induce, to some extent, a phenotype change in MCF-7 cells are in agreement with previous reports. It was notably shown that the stable transfection of PKCα in these cells produced dramatic morphologic alterations including loss of an epithelioid appearance, a significant reduction in ERα expression, and a decrease in estrogen-dependent gene expression. A significant increase in the level of the fibroblastic/mesenchymal marker vimentin was also observed (5). Many genes regulated by PMA were associated to specific cell properties or features. Only a few examples will be given here.

A series of genes were related to cell adhesion and cell architecture. Most were up-regulated by PMA, and their identity supports a profound effect of PKC activation on actin fiber dynamics, cell spreading and motility, and signaling from the extra-cellular matrix.

Thus, several genes coding for extra-cellular matrix components were increased: *FN1, LAMB3, MFGE8* (milk fat globule-EGF factor 8 protein), *MGP* (matrix Gla protein). While some genes coding for cell adhesion molecules, notably integrins components, were increased (*ICAM1, ITGA3, ITGA5*), other were decreased (*DSCAM*, down syndrome cell adhesion molecule; *ITGAE*, integrin α E). The actin γ1 gene (*ACTG1*), was up-regulated, while genes encoding actin fiber-organizing proteins were either increased (*ACTN1*, actinin α 1; *VIL2*, villin 2) or decreased (*EPLIN*, epithelial protein lost in neoplasm ß). *KRT1* (keratin 1), a component of intermediate filaments, was increased. Focal adhesion plaques are structures that form at the ends of actin fibers and serve as sites of force transmission. The integrins are present at these plaques and connect extra-cellular matrix components to actin filaments, through the action of many actin-attachment proteins and other cytoplasmic adhesion proteins. Two components of focal adhesion plaques, *SDC4* (syndecan 4) and *ZYX* (zyxin) were up-regulated, as was *CAPN2* (calpain-2), which modulates zyxin-actinin relations.

One interesting feature was that all the modulated genes identified as participating in proteolysis and proteolysis inhibition were up-regulated by PMA. These included *CSTB* (cystatin B), *CTSD* (cathepsin D), *CTSL, MMP1, MMP9, MMP10, PLAUR* (plasminogen activator, urokinase receptor), *PRSS15* (protease, serine, 15), *SERPINA3* (serine (or cysteine) proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 3), *SERPINB8* [serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8]. This might provide to the cells the potential to rapidly and profoundly process proteins composing their extra-cellular environment. The amount of such proteins is generally higher around mesenchymal cells normally embedded in a dense extra-cellular matrix than around epithelial cells.

Table III. Genes regulated by PMA in both MCF-7 and MDA-MB-231 BCC.

Description	Gene name	GB Acc	MCF-7	MDA-MB-231
Matrix metalloproteinase 10 (stromelysin 2)	MMP10	NM_002425	18.76	3.03
Nuclear receptor subfamily 4, group A, member 1	NR4A1	XM_006843	6.79	2.15
Centromere protein A (17 kDa)	CENPA	AL555786	0.28	0.45
Cell division cycle 2	CDC2	NT_008583	0.23	0.46
Epithelial V-like antigen 1	EVA1	AF275945	4.08	0.44
Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	ID1	AL117381	2.87	0.34

Genes modulated by PMA in both MCF-7 and MDA-MB-231 cells. Six genes were modulated by PMA in both MCF-7 and MDA-MB-231 cells. They are listed in Table III. *CDC2* (cell division cycle 2) and *CENPA* (centromere protein A) are know to be cell cycle-associated, culminating in G_2/M phase. Their observed down-regulation in both cell lines is in agreement with the previously described growth-arrest effects of PMA (see *e.g.* ref. 11). *NR4A1* (nuclear receptor subfamily 4, group A, member 1) was up-regulated in both cell lines. Its protein product is also known as Nur-77 or NGFI-B (nerve growth factor-induced clone B). *NR4A1* is an immediate early response gene for a number of growth factors signaling pathways and has been implicated in cell proliferation, differentiation, and apoptosis. Its induction by PMA has been previously described in MCF-7 (14), but not in MDA-MB-231 cells. In gastric cancer cells, PMA has been shown not only to up-regulate the *NR4A1* mRNA level, but also to lead to translocation of NR4A1 protein from the nucleus to the mitochondria, which causes the release of cytochrome c and the triggering of apoptosis (15). Whether this may occur in PMA-treated BCC remains to be established. *MMP10* encodes a member of the stromelysin family of matrix metalloproteinases, which have a broad substrate specificity and may notably degrade several extra-cellular matrix (ECM) components (laminin, fibronectin, non-fibrillar collagens) (16). MMP10 is specifically expressed in epithelial cells and has previously been detected in MCF-7 and MDA-MB-231 BCC (17,18). Strong MMP10 expression has been observed in breast carcinomas, especially in the ECM adjacent to blood vessels (19). As two PMA-responsive, AP-1 transcription factor-binding sites are present in the *MMP10* promoter (20), they could mediate the induction of this gene by the phorbol ester in both MCF-7 and MDA-MB-231. This induction is expected to give to the BCC a higher ability to degrade ECM.

Three genes were regulated in an opposite way in MCF-7 and MDA-MB-231 BCC: *ID1* and *EVA1*. *ID1* was up-regulated by PMA in MCF-7, while it was and down-regulated in MDA-MB-231 cells. Basic loop-helix-loop (bHLH) transcription factors regulate the differentiation programs in various cells. The Id family of proteins (comprised of four members designated Id1-Id4) does not possess a basic DNA binding domain and function as dominant-negative regulator of bHLH proteins by hetero-dimerizing with the latter. In BCC, Id1 has been associated with proliferation, apoptosis, and pheno-type definition (21). It has been shown that *ID1* is constitutively expressed at a high level in ERα-negative, fibroblast-like MDA-MB-231 and MDA-MB-435 BCC, but not in ERα-positive, luminal epithelial-like MCF-7 and T-47D cells and, in general, in normal luminal epithelial cells of mammary glands (22). In T-47D cells, it is, however, induced by serum and estrogens, but repressed by progesterone (23). An *ID1* cDNA, constitutively expressed in the non-aggressive T-47D cells, conferred to these cells a more aggressive phenotype, as measured by growth and invasiveness (23). Our data suggest that Id1 could play a role in the PMA-mediated induction of a more fibroblast-like or 'dedifferentiated' phenotype in the luminal epithelial-like MCF-7, while its down-regulation could attenuate, to some extent, the expression of this fibroblast-like phenotype in MDA-MB-231 cells. This latter effect, however, is unlikely to be extensive, considering that only a few genes appeared to be modulated by PMA in these cells. *EVA1* encodes a transmembrane glycoprotein, (epithelial V-like antigen) expressed in various epithelia and first identified as homotypic adhesion molecule involved in thymus histogenesis (24). To our knowledge, its role in breast cancer has not been extensively studied. Its modulation could alter the cell-cell and cell-environment interactions in breast tumors.

In conclusion, our micro-array-based study supports previous observations indicating that the BCC response to the PKC-activator PMA is variable. The ERα-positive, luminal epithelial-like, weakly invasive MCF-7 appeared much more responsive to the phorbol ester than the ERα-negative, fibroblast-like, highly aggressive MDA-MB-231 cells. Our study revealed genetic changes consistent with profound effects of PMA on MCF-7 cell growth, architecture, and interactions with their environment.

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