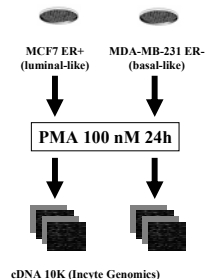


## Abstract

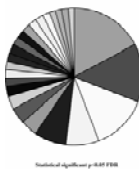
We have studied the effects of the protein kinase C (PKC)-activator phorbol 12-myristate 13-acetate (PMA) on gene expression in two highly different breast cancer cell (BCC) lines, the estrogen receptor alpha (ER)-positive, "luminal epithelial-like" MCF-7 and the ER-negative, "fibroblast-like" MDA-MB-231. These express constitutively low and high PKC activities, respectively. After a 24 h exposition to 100 nM PMA, the number of genes showing a altered expression at the 2 fold change level was much higher in MCF-7 (435) than in MDA-MB-231 (18) BCC. Three of these genes, namely *CDCC2*, *CENPA*, *MMP10*, were altered in the same way in both cell lines. Two genes were regulated in an opposite way: *IDI1* and *EVAL1*. Many of the genes modulated in MCF-7 BCC (about 22% of total) were cell-cycle associated; they were down-regulated. The ER gene, *ESR1*, and a series of other genes associated to the ER-positive, "luminal epithelial-like" phenotype of BCC were down regulated, while genes related to a more "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, and on the interactions between BCC and their neighboring proteins. Of note, all the genes identified as related to proteolysis and their controls were up regulated. In summary, PMA effects suggest that PKC activation may induce, to some extent, a more "fibroblast-like" phenotype in the (ER)-positive, "luminal epithelial-like" MCF-7 BCC and significantly modulate the interactions of these cells with their environment.

## Material and Methods



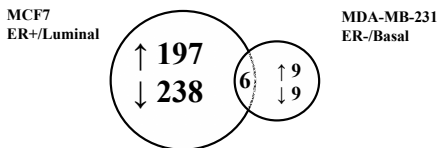
Two (forward and reverse fluorochrome) hybridizations for each experimental condition were performed

## Molecular Function



other (202 genes : sum of groups which include a max of 1 or 2 genes per function)

## Venn Logic Diagram

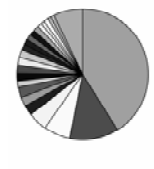


Venn diagram using genes with differential expression of at least 2-fold in two reciprocal fluorochrome experiments in MCF-7 and MDA-MB-231 BCC treated with PMA.

Functional profiles (using Gene Ontology terms) for biochemical function, biological process, cellular role, cellular component, molecular function, chromosome location (Onto-Express).

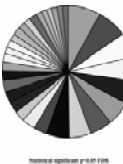
- ATP binding (35)
- DNA binding (28)
- hydrolytic activity (26)
- receptor activity (16)
- protein serine/threonine kinase activity (15)
- structural molecule activity (8)
- protein tyrosine kinase activity (8)
- nucleotide binding (6)
- apoptosis inhibitor activity (5)
- DNA dependent ATPase activity (4)
- protein phosphatase type 2C activity (4)
- myosin phosphatase activity (4)
- transcription-dependent protein serine/threonine phosphatase activity (4)
- calcium-dependent protein serine/threonine phosphatase activity (4)
- CTD phosphatase activity (4)
- serp-1 serpin domain DNA binding (4)
- protein phosphatase type 2A activity (3)
- protein kinase activity (4)
- cell adhesion receptor activity (4)
- glucocorticoid and/or androgen activity (3)
- delta DNA polymerase activity (3)
- cyclic-dependent protein kinase inhibitor activity (3)
- adenosine triphosphatase activity (3)
- damaged DNA binding (3)

## Cellular Component



other (102 genes : sum of groups which include a max of 1 or 2 genes per function)

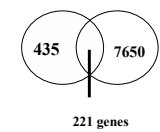
## Biological Process



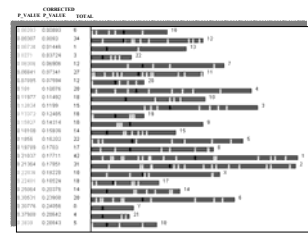
other (113 genes : sum of groups which include a max of 1 or 2 genes per process)

- DNA replication (18)
- regulation of cell cycle (16)
- DNA repair (14)
- transcription (13)
- apoptosis (13)
- immune response (13)
- cell cycle (12)
- cell-cell signaling (12)
- biological process (12)
- metabolism (12)
- cell matrix adhesion (7)
- anti-apoptosis (6)
- cytokinesis (6)
- vision (6)
- skelatal development (6)
- protein acid activity (6)
- cell cycle arrest (4)
- regulation of ERK activity (4)
- cell cycle arrest (4)
- blood coagulation (4)
- DNA dependent DNA replication (4)
- metabolic chromosome condensation (4)
- nucleotide biosynthesis (4)
- cell shape and cell size control (4)
- integrin-mediated signaling pathway (3)
- chromosome organization and biogenesis (term Eukaryote) (3)

Overlap between PMA regulated genes and NCI 7650 probe-Elements Microarray  
C. Sotiriou et al. Proc Natl Acad Sci U S A. 2003 Sep 2; 100(18):10374-9.



## Chromosomal Information



## Hierarchical cluster diagram of 99 BC specimens based on the 221 PMA-regulated genes



## Conclusions

- The number of modulated genes after PMA are higher in MCF7/ER+ BCC compared to MDA-MB-231 BCC lines.
- PMA effects suggest that PKC activation may induce, to some extent, a more "fibroblast-like" phenotype in the (ER)-positive, "luminal epithelial-like" MCF-7 BCC and significantly modulate the interactions of these cells with their environment.