# Identification of genes involved in growth inhibition of breast cancer cells transduced with estrogen receptor

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Received 25 June 2003; revised 16 September 2003; accepted 19 September 2003

First published online 2 October 2003

Edited by Robert Barouki

**Abstract Estrogen receptor α (ERα)-negative breast cancer cells display an aggressive phenotype. We previously showed that adenoviral expression of ERα in ER-negative breast cancer cells leads to an estrogen-dependent down-regulation of the proliferation, which could be of interest to control the growth of such cells. In this study, we observed an increase in protein levels of p21 and p27 cyclin-dependent kinase inhibitors, whereas pRb phosphorylation is strongly decreased. Flow cytometry experiments showed a slower transit of cells in G1 (hormone-independent), a hormone-induced accelerated transit through S phase and a possible arrest in G2/M phase. In addition, ERα-expressing cells were undergoing apoptosis. By using cDNA macroarrays, we identified a novel collection of genes regulated by liganded ERα potentially regulating cell cycle, apoptosis, cell signalling, stress response and DNA repair.**


*Key words:* Estrogen; Receptor; Proliferation; cDNA array

## 1. Introduction

Breast cancer is one of the leading causes of premature death in women in western countries [1]. Increased lifetime exposure to estrogens is an established risk factor for development of breast cancer. Estrogen action is mediated by two estrogen receptors, ERα and ERβ. ERβ is poorly expressed in breast tumors [2]. A high percentage of early stage mammary tumors are ERα-positive and about 50% of these patients respond to anti-estrogen or endocrine therapy [3,4]. Later stages of breast cancer are more aggressive and refractory to most therapies and this correlates with the ERα-negative phenotype of these tumors [5]. ERα-positive tumor cells are poorly metastatic in nude mice when compared with some ERα-negative breast cancer cells [6]. In patients, ERα-positive tumors are more differentiated and have lower metastatic potential than ERα-negative tumors [7], suggesting a protective role of ERα in tumor progression. Introduction of ERα into ER-negative breast cancer cells has been thought to provide a rational basis for converting anti-estrogen-resistant cells to hormonal manipulation. However, in contrast to the expected stimulated proliferation of ER-positive cells by estradiol, the restoration of ERα expression in ER-negative breast cancer cells leads to a ligand-dependent inhibition of proliferation as shown by numerous studies [8–11]. The mechanisms underlying this phenomenon have been poorly investigated and could be of great interest to control the proliferation of these aggressive types of tumor cells. The goal of this study was to discern events responsible for the ligand-dependent inhibition of breast cancer cells expressing exogenously ERα by analyzing possible cell cycle regulation and modulation of gene expression.

## 2. Materials and methods

### 2.1. Recombinant adenovirus construction and propagation

The complete coding sequence of wild-type hERα cDNA was subcloned in *Bam*HI site of the pACsk12CMV5 shuttle vector. To obtain recombinant viruses, permissive HEK-293 cells (human embryonic kidney cells) were cotransfected with the backbone or recombinant pACsk12CMV5-hER plasmid and with pJM17, which contains the remainder of the adenoviral genome as previously described [11]. In vivo recombination of the plasmids generates infectious viral particles Ad5 (backbone virus) and Ad-hERα. Titered virus stocks were used to infect MDA-MB-231 cells.

### 2.2. Cell culture and infection

MDA-MB-231 cells were maintained in Leibovitz L-15 medium supplemented with 10% fetal calf serum (FCS) and gentamicin. To wean the cells off steroids, they were cultured in phenol red-free Dulbecco's modified Eagle's medium/F12 supplemented with 10% charcoal dextran-treated FCS for 4 days. For infection, cells were cultured in the same medium and infected with Ad5 or Ad-hERα viruses at a multiplicity of infection of 100.

### 2.3. Whole cell extract preparation and Western blot

Cell extracts were prepared in NP40 buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, 1% NP40, 10 μM sodium fluoride, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, leupeptin, pepstatin A and 20 nM okadaic acid). Cells were washed twice in ice-cold phosphate-buffered saline (PBS), scraped in ice-cold NP40 buffer and lysed for 10 min. Cell lysates were cleared by a 15 min centrifugation at 4°C, protein content in the supernatant was assayed by the Bradford protein assay (Bio-Rad). The cell extracts were diluted 1:1 in 2×Laemmli sample buffer, followed by boiling for 5 min. Equal amounts of protein were loaded and separated for sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel. Proteins were transferred to cellulose nitrate filters (Schleicher and Schuell, Germany) and blocked for 30 min at 37°C in 5% non-fat milk in TBST (Tris-buffered saline with 0.1% Tween 20). Membranes were incubated with the primary antibody for 1 h at room temperature in TBST. The primary antibodies were: cdc2 (Ab-4; NeoMarkers)

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0014-5793/03/$22.00 
doi:10.1016/S0014-5793(03)01090-1

1:500; cdk2 (Transduction Laboratories) 1:1000; Cyc A (BF683; Santa Cruz Biotechnology) 1:200; Cyc B1 (GNS1; Santa Cruz Biotechnology) 1:200; Cyc D1 (CDS6, Sigma) 1:200; Cyc D3 (Transduction Laboratories) 1:1000; Cyc E (M20; Santa Cruz Biotechnology) 1:200; ERα (ER-311 [12]) 1:1000; ERK (K23; Santa Cruz Biotechnology) 100 μg/ml; Rb (IF8; Santa Cruz Biotechnology) 1:500; $p21^{CIP-1}$ (L17; Santa Cruz Biotechnology) 1:100; $p27^{Kip-1}$ (C19; Santa Cruz Biotechnology) 1:100. After washing, horseradish peroxidase-linked secondary antibodies (Amersham Pharmacia) were added and target protein bands were detected using ECL (Amersham Pharmacia).

### 2.4. Flow cytometry experiments

To analyze the effects of recombinant viruses on the cell cycle, MCF-7 cells were infected with the adenoviral vectors, and cells were fixed in 75% ethanol for 2 min. Fixed cells were then stained with PBS containing 40 μg/ml propidium iodide and 100 μg/ml RNase. After a 30 min incubation at 37°C, analysis was performed on an Epics-XL flow cytometer (Beckman Coulter, Fullerton, CA, USA) and analyzed with Modfit software (Verity Software, Topsham, ME, USA).

### 2.5. RNA extraction, Northern blot and cDNA microarrays

Total RNA was isolated with TRIzol reagent (InVitrogen) as described by the manufacturer. RNA quantity was determined photometrically by absorption at 260 nm and quality was checked by examination of the 28S and 18S rRNA bands in ethidium bromide-stained agarose gels. After two phenol/chloroform extractions, RNA was precipitated. To remove genomic DNA contamination, RNA was treated with RNase-free DNase I (Clontech, Palo Alto, CA, USA), and was then dissolved in RNase-free $H_2O$ and stored at −80°C until analysis. For Northern blot analysis, 20 μg RNA was electrophoresed and then hybridized with the different probes. The Atlas human macroarray (7740-1: 588 genes) was purchased from Clontech. For cDNA probes, 5 μg of total RNA from MDA-MB-231 infected with Ad-hERα and treated or not for 24 h with E2 ($10^{-8}$ M) was used. The Atlas arrays were then hybridized with the probes according to the manufacturer's instructions. Array images were analyzed using Atlas Image 2.0 software (Clontech). The cut-off for cDNA array screening was set to 2.0-fold change for up-regulated genes and to 0.4-fold change (60% decrease) for down-regulated genes.

## 3. Results

### 3.1. Growth inhibition involves G2/M transition blockage

We have previously shown that exogenous expression of ERα in MDA-MB-231 ER-negative breast cancer cells leads to growth inhibition in the presence of estradiol [11]. To better characterize the phenomenon involved in growth inhibition, we performed flow cytometry experiments on cells which had been treated for different times with estradiol (Fig. 1). Cell cycle distribution of Ad5-infected cells remained unchanged upon estrogen treatment. On the other hand, introduction of hERα in MDA-MB-231 cells led to both ligand-independent and ligand-dependent changes in the cell cycle. An accumulation of cells in G1 phase was observed in a ligand-independent manner in hERα-expressing cells compared to Ad5-infected cells. Upon treatment with estradiol, the proportion of ERα-positive cells in S phase was strongly diminished (from 27 to 16%). The proportion of cells in G2/M phase, which was strongly reduced after ERα introduction, increased from 13 to 17% following E2 addition. Importantly, we also detected the presence of a sub-G1 peak in Ad-hERα cells (representing around 1.5% of total cells in the absence of estradiol), which is characteristic of cells undergoing apoptosis (Fig. 1). This peak reached its maximum in Ad-hERα-infected cells treated for 4 h with E2, with 3.8% apoptotic cells, whereas Ad5-infected cells displayed less than 0.5% apoptotic cells.

### 3.2. Analysis of cell cycle modulator expression

To dissect the mechanisms underlying this inhibition of proliferation, we analyzed at the protein level the expression of a collection of known cell cycle modulators. A time course treatment with estradiol was performed on Ad5- or Ad-hERα-infected cells and whole cell extracts were analyzed by Western blot (Fig. 2). The presence of ERα was only detected in Ad-hERα-infected cells. In addition, the appearance of a slower migrating band upon estradiol treatment could correspond to a phosphorylated form of ERα. We observed both ligand-dependent and ligand-independent changes in cycle modulator expression. Cyclins D1 and E were up-regulated and cyclin A down-regulated in a ligand-independent manner. ERK phosphorylation did not show significant modification, whereas pRb phosphorylation was strongly diminished in a ligand-dependent manner. The cyclin-dependent kinase inhibitors (CDKIs) p21 and p27 showed a nice up-regulation following estradiol treatment. Finally, cyclin D3, cdc2 and cdk2 levels were stable. In summary, these data show a complex regulation of cyclins and CDKI involved in G1 to S phase transition.

### 3.3. Liganded ERα induces a complex pattern of gene regulation

To further characterize the mechanisms underlying the growth inhibitory effect of liganded ERα, we compared the expression of 588 genes between ERα-infected cells treated or not with E2, by using cDNA macroarrays. A total of 13 genes were up-regulated by E2 (Table 1), whereas 26 genes were down-regulated by E2 (Table 2). The genes identified were potentially involved in oncogenesis or tumor suppression (RAF, c-myc, c-fms, c-jun, Fra-1, Axl), cell cycle (cyclin G2,

Table 1
Genes up-regulated by E2 in Ad-hERα-infected MDA-MB-231 cells

| GenBank accession number | Gene name | Fold change | Putative function |
|---|---|---|---|
| X03484 | RAF | 2.4 | Oncogenes – tumor suppressors/intracellular kinase network members |
| U47414 | Cyclin G2 | 2.3 | Cell cycle/cyclins |
| D38305 | TOB | 3.1 | Cell cycle/adapters and receptor-associated proteins |
| U15174 | BNIP3 | 3.2 | Apoptosis/Bcl2 family proteins |
| S40706 | GADD153 | 2.1 | Apoptosis/DNA damage |
| U09579 | p21/CIP-1 | 15.8 | Cell cycle/CDK inhibitors |
| U30504 | TAFII31 | 2.0 | Transcription/RNA polymerase |
| K03222 | TGFα | 11.4 | Cell signalling – extracellular communication proteins/growth factors |
| X03438 | G-CSF | 12.3 | Cell signalling – extracellular communication proteins/cytokines |
| M16552 | Thrombomodulin | 6.2 | Cell signalling – extracellular communication proteins/growth factors |
| D30751 | BMP-4 | 4.8 | Cell signalling – extracellular communication proteins/growth factors |
| X70326 | Macmarcks | 2.9 | Intracellular transducers/kinase activators and inhibitors |
| K02770 | IL-1β | 18.5 | Cell signalling – extracellular communication proteins/cytokines |

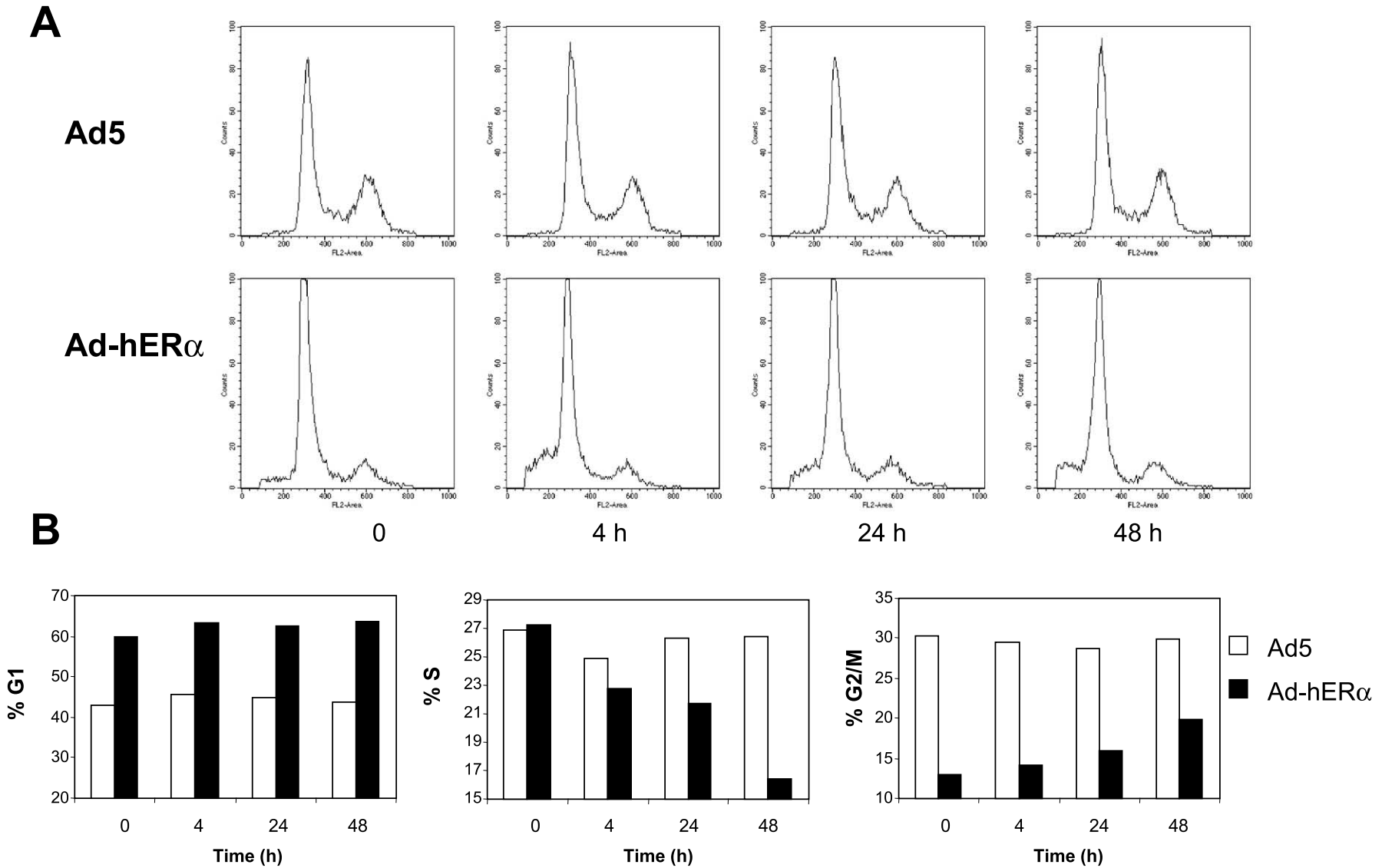


Fig. 1. ERα blocks cell cycle. Ad5- or Ad-hERα-infected MDA-MB-231 cells which had been treated for 0, 4, 24 or 48 h with E2 $10^{-8}$ M were analyzed by fluorescence-activated cell sorting (FACS) for cell cycle distribution. A: Representative profiles of FACS analysis. B: Quantification of cell cycle distribution.

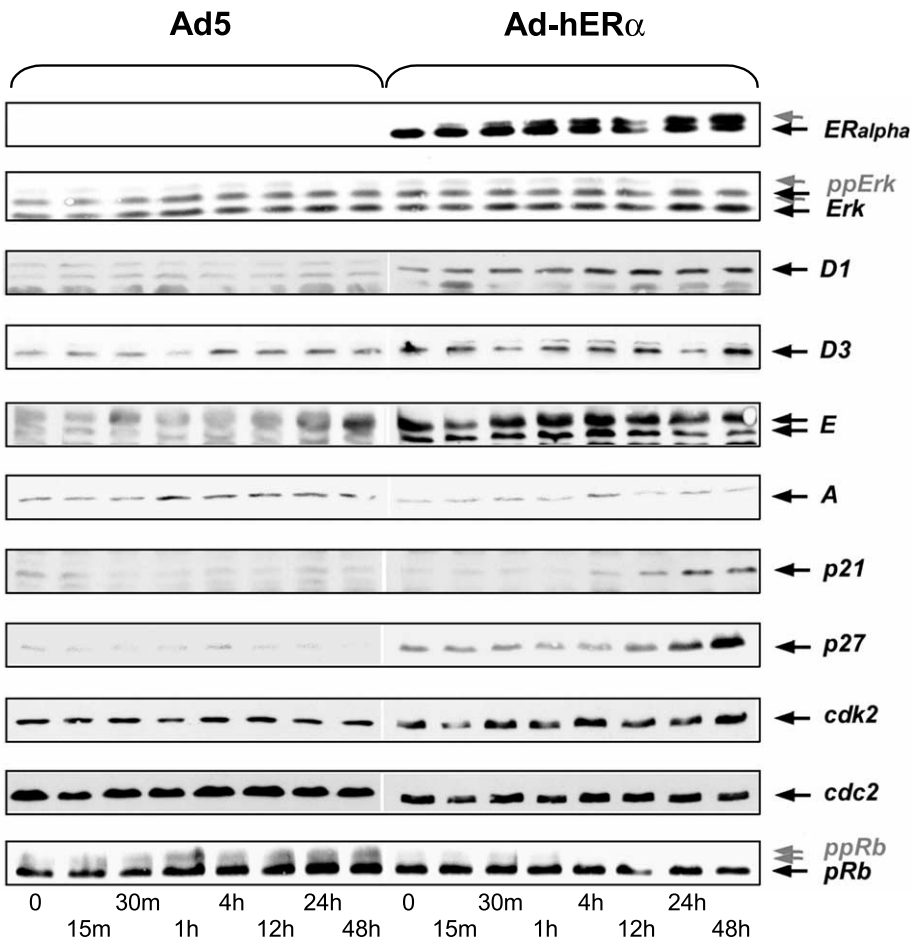


Fig. 2. Modulation of cell cycle regulatory factors by ERα. Whole cell extracts from Ad5- or Ad-hERα-infected MDA-MB-231 cells which had been treated for 0–48 h with E2 ($10^{-8}$ M) were used for determination of the expression of ERα, Erk, cyclin D1, cyclin D3, cyclin E, cyclin A, $p21^{CIP-1}$, $p27^{Kip-1}$, cdk2, cdc2 and pRb by Western blot.

Table 2
Genes down-regulated by E2 in Ad-hERα-infected MDA-MB-231 cells

| GenBank accession number | Gene name | Fold change | Putative function |
|---|---|---|---|
| V00568 | c-myc | 0.2 | Oncogenes – tumor suppressors/transcription activators and repressors |
| X03663 | c-fms | 0.3 | Oncogenes – tumor suppressors/intracellular transducers |
| J04111 | c-jun | 0.1 | Oncogenes – tumor suppressors/transcription activators and repressors |
| X16707 | Fra-1 | 0.1 | Oncogenes – tumor suppressors/transcription activators and repressors |
| M76125 | Axl | 0.6 | Oncogenes – tumor suppressors/intracellular transducers |
| U02687 | STK-1 | 0.5 | Cell cycle/intracellular transducers |
| M62424 | Thrombin receptor | 0.3 | Intracellular transducers/growth factor receptors |
| M31630 | ATF-2 | 0.5 | Transcription activators and repressors/intracellular transducers |
| M34664 | HSP60 | 0.6 | Stress response proteins/heat shock proteins |
| X15722 | Glutathione reductase | 0.1 | Stress response proteins/xenobiotic transporters |
| M21304 | Glutathione peroxidase (GPX1) | 0.4 | Stress response proteins/xenobiotic transporters |
| U18321 | DAP-3 | 0.6 | Apoptosis |
| J04088 | TopoIIα | 0.2 | DNA synthesis – recombination and repair/topoisomerases |
| D21235 | HHR23A | 0.4 | DNA synthesis – recombination and repair/nucleotide excision repair |
| D21090 | HHR23B | 0.4 | DNA synthesis – recombination and repair/nucleotide excision repair |
| M60974 | Gadd45 | 0.5 | Apoptosis/DNA synthesis – recombination and repair |
| U35835 | DNA-PK | 0.4 | DNA synthesis – recombination and repair/stress response proteins |
| L34673 | HIP116 | 0.2 | Transcription/basic transcription factors |
| D90209 | ATF-4 | 0.2 | Transcription/transcription activators and repressors |
| M28372 | CNBP | 0.1 | Transcription/basic transcription factors |
| M83234 | Y-box protein | 0.4 | Transcription/basic transcription factors |
| M59911 | Integrin α-3 chain | 0.6 | Cell adhesion proteins/cell–cell adhesion receptors |
| X07979 | Integrin β1 subunit | 0.4 | Cell adhesion proteins/cell–cell adhesion receptors |
| M92934 | CTGF | 0.1 | Cell signalling – extracellular communication proteins/growth factors |
| M14200 | DBI/ACBP | 0.6 | Metabolism/nucleotide metabolism |
| M31159 | IGFBP-3 | 0.4 | Cell signalling – extracellular communication proteins/hormones |

TOB, p21, STK-1), apoptosis (BNIP3, Gadd45, Gadd153, DAP-3), transcription (TAFII31, ATF-2, ATF-4, HIP116, CNBP, Y box), cell signalling (TGFα, G-CSF, thrombomodulin, BMP-4, IL-1β, thrombin receptor, Macmarcks, CTGF), cell adhesion (integrin α3 chain, integrin β1 subunit), stress response (HSP60, glutathione reductase, glutathione peroxidase), DNA synthesis and repair (TopoIIα, HHR23A, HHR23B, DNA-PK, DBI). This suggests that exogenous expression of ERα and subsequent activation by E2 lead to major changes in gene regulation of the cells affecting multiple aspects of cell life.

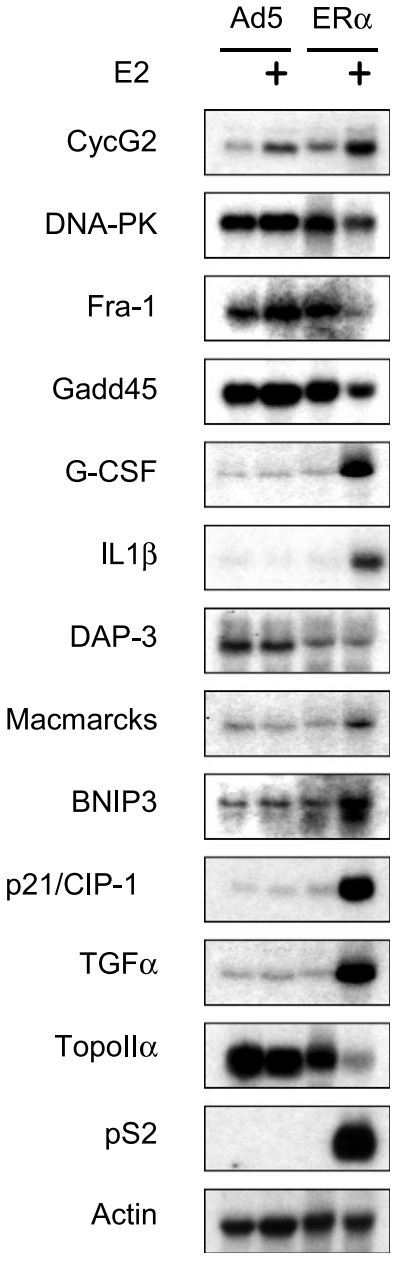


Fig. 3. Analysis of a subset of genes regulated by ERα in MDA-MB-231 cells. The expression of cyclin G2 DNA-PK, Fra-1, Gadd45, G-CSF, IL-1β, DAP-3, Macmarcks, BNIP3, $p21^{CIP-1}$, TGFα, TopoIIα and pS2 was analyzed by Northern blot using RNA from Ad5- or Ad-hERα-infected MDA-MB-231 cells, treated or not for 48 h with E2.

### 3.4. Confirmation of the regulations by Northern blot

To confirm the regulations identified by cDNA array screening, we analyzed the expression of 13 genes by Northern blot (Fig. 3). The pS2 gene (not present on the array), which is a marker of estrogen receptor function [13], was used as a positive control of estrogen regulation and expressed only in Ad-ERα-infected cells treated with E2. DAP-3 levels were down-regulated by ERα in a ligand-independent manner. We observed that the levels of Fra-1, Gadd45 and TopoIIα and to a minor extent DNA-PK were effectively down-regulated by liganded-ERα, whereas G-CSF, IL-1β, Macmarcks, BNIP3, p21/CIP-1 and TGFα levels were up-regulated, which validates our screen. Cyclin G2 was up-regulated by E2 in ERα-expressing cells and to a minor extent in Ad5-infected cells. This could be due to the low levels of endogenous ERα and ERβ in these cells.

## 4. Discussion

To identify the mechanisms underlying cell growth inhibition triggered by liganded ERα in ER-negative breast cancer cells, we have analyzed cell cycle distribution and gene expression changes. Our results suggest that introduction of ERα in ER-negative cells leads to both ligand-independent and ligand-dependent modifications. Expression of exogenous ERα induces changes in cell cycle kinetics characterized by a slower transit of cells in G1 (independent of hormone) and an accelerated cell exit from the S phase (hormone-induced) with a possible bottleneck of the cycle in G2/M. Apoptosis, on the other hand, appears to be promoted by hormone-activated ER. We should point out that previous work from our group [11] has shown an increase of cell size, when they were infected by Ad-hERα and treated with E2. This could reflect cell blockage in G2/M and appearance of cells with high DNA content.

Ligand-dependent modification of the cell cycle is concomitant with an up-regulation of p21, p27, Macmarcks and TOB levels, a down-regulation of STK1 expression and a dephosphorylation of pRb. p21, p27, TOB, Macmarcks and cyclin G2 have been shown to inhibit proliferation [14–17], whereas STK1 overexpression could increase proliferation [18]. The down-regulation of Gadd45, whose expression is frequently increased in growth-arrested cells and regulated by BRCA-1 [19], might be explained by the down-regulation of BRCA-1 we observed previously in such infected cells [20]. On the other hand, apoptosis is occurring as shown by the appearance of a sub-G1 peak and induction of pro-apoptotic BNIP3 [21]. It is also interesting to note that several oncogenes such as c-myc, c-fms, c-jun, Fra-1, Axl, or transcription factors such as ATF-2, ATF-4, HIP116, CNBP or Y box are down-regulated. All these proteins generally promote tumorigenesis [22–27]. Abnormal expression of c-fms, Axl, members of the AP-1 family, CNBP (which stimulates c-myc promoter activity) or Y box protein by malignant cells is correlated with a poor prognosis [22–26,28].

Down-regulation of topoisomerase IIα, HHR23A, HHR23B, DBI/ACBP and to a lesser extent DNA-PK suggests that reintroduction of ERα in MDA-MB-231 cells leads to a decreased efficiency of DNA repair [29–31], which could eventually lead to apoptosis. In addition to the weaker ability of the cells to defend themselves against DNA damage, it is likely that the cells are also losing their ability to respond to

stress signals as shown by down-regulation of HSP60, glutathione peroxidase and glutathione reductase [32,33].

Overall changes in integrin and BMP-4 and ligand-independent down-regulation of DAP-3 [34–36] could also account for the previously observed inhibition of invasion observed after reintroduction of ERα [9]. Other events such as down-regulation of thrombin receptor and up-regulation of thrombomodulin could also account for the decreased invasiveness of these cells [37–39]. In addition, the down-regulation of CTGF, which promotes angiogenesis [40], suggests that ERα exogenous expression could reduce the angiogenesis events during tumor formation. It is also interesting to note that the majority of the genes regulated by E2 that we discovered have not been previously identified as potential targets of ER, confirming the importance of our screen.

In conclusion, our data suggest that reintroduction of ERα in ER-negative breast cancer cells could be a valuable strategy to limit their growth and their invasion. This is also concomitant with a reduced ability to repair damaged DNA and to respond to stress signals, which further suggests that the cells are more likely to lose their advantages over normal cells.

*Acknowledgements:* We thank the Vector Core of the University Hospital of Nantes supported by the Association Française contre les Myopathies (AFM) for the production of adenovirus. This work was supported by grants from ARC (Association pour la Recherche contre le Cancer, Grant 4302), la Ligue Nationale contre le Cancer (Comité du Gard), INSERM, Associazione Italiana per la Ricerca sul Cancro (Grants 2001–02), Ministerio d'Istruzione, Università e Ricerca of Italy (Grants PRIN 2002067514_002 and FIRB RBNE0157EH_001). We are grateful to C. Duperray for his help in FACS studies.

## References

[1] Kelsey, J.L. and Berkowitz, G.S. (1988) Cancer Res. 48, 5615–5623.
[2] Roger, P., Sahla, M.E., Makela, S., Gustafsson, J.A., Baldet, P. and Rochefort, H. (2001) Cancer Res. 61, 2537–2541.
[3] Santen, R., Manni, A., Harvey, H. and Redmond, C. (1990) Endocr. Rev. 11, 221–265.
[4] Jordan, V.C., Jeng, M.H., Catherino, W.H. and Parker, C.J. (1993) Cancer 71, 1501–1505.
[5] Dickson, R.B. and Lippman, M.E. (1995) Endocr. Rev. 16, 559–589.
[6] Osborne, C.K., Hobbs, K. and Clark, G.M. (1985) Cancer Res. 45, 584–590.
[7] McGuire, W.L. (1975) Cancer 36, 638–644.
[8] Jiang, S.Y. and Jordan, V.C. (1992) J. Natl. Cancer Inst. 84, 580–591.
[9] Platet, N., Cunat, S., Chalbos, D., Rochefort, H. and Garcia, M. (2000) Mol. Endocrinol. 14, 999–1009.
[10] Garcia, M., Derocq, D., Freiss, G. and Rochefort, H. (1992) Proc. Natl. Acad. Sci. USA 89, 11538–11542.
[11] Lazennec, G., Bresson, D., Lucas, A., Chauveau, C. and Vignon, F. (2001) Endocrinology 142, 4120–4130.
[12] Abbondanza, C., de Falco, A., Nigro, V., Medici, N., Armetta, I., Molinari, A.M., Moncharmont, B. and Puca, G.A. (1993) Steroids 58, 4–12.
[13] Masiakowski, P., Breathnach, R., Bloch, J., Gannon, F., Krust, A. and Chambon, P. (1982) Nucleic Acids Res. 10, 7895–7903.
[14] Craig, C. et al. (1997) Oncogene 14, 2283–2289.
[15] Jin Cho, S., La, M., Ahn, J.K., Meadows, G.G. and Joe, C.O. (2001) Biochem. Biophys. Res. Commun. 283, 273–277.
[16] Ramsden, J.J. (2000) Int. J. Biochem. Cell Biol. 32, 475–479.
[17] Tian, Y.L., Liu, F.R., Liu, J., Jiang, L., Luo, Y. and Zhang, X. (2002) Ai Zheng 21, 577–581.
[18] Carow, C.E. et al. (1996) Blood 87, 1089–1096.
[19] Mullan, P.B. et al. (2001) Oncogene 20, 6123–6131.
[20] Lazennec, G. and Katzenellenbogen, B.S. (1999) Mol. Cell. Endocrinol. 149, 93–105.
[21] Chen, G. et al. (1999) J. Biol. Chem. 274, 7–10.
[22] Berclaz, G., Altermatt, H.J., Rohrbach, V., Kieffer, I., Dreher, E. and Andres, A.C. (2001) Ann. Oncol. 12, 819–824.
[23] Kacinski, B.M. et al. (1991) Oncogene 6, 941–952.
[24] Shaulian, E. and Karin, M. (2001) Oncogene 20, 2390–2400.
[25] Shimizu, K., Chen, W., Ashique, A.M., Moroi, R. and Li, Y.P. (2003) Gene 307, 51–62.
[26] Oda, Y. et al. (2003) J. Pathol. 199, 251–258.
[27] Hermeking, H. (2003) Curr. Cancer Drug Targets 3, 173–175.
[28] Bamberger, A.M., Methner, C., Lisboa, B.W., Stadtler, C., Schulte, H.M., Loning, T. and Milde-Langosch, K. (1999) Int. J. Cancer 84, 533–538.
[29] Sugasawa, K., Masutani, C., Uchida, A., Maekawa, T., van der Spek, P.J., Bootsma, D., Hoeijmakers, J.H. and Hanaoka, F. (1996) Mol. Cell. Biol. 16, 4852–4861.
[30] Villman, K., Stahl, E., Liljegren, G., Tidefelt, U. and Karlsson, M.G. (2002) Mod. Pathol. 15, 486–491.
[31] Pucci, S. et al. (2001) Oncogene 20, 739–747.
[32] Perquin, M., Oster, T., Maul, A., Froment, N., Untereiner, M. and Bagrel, D. (2000) Cancer Lett. 158, 7–16.
[33] Cornford, P.A. et al. (2000) Cancer Res. 60, 7099–7105.
[34] Mariani, L. et al. (2001) Clin. Cancer Res. 7, 2480–2489.
[35] van den Wijngaard, A., Mulder, W.R., Dijkema, R., Boersma, C.J., Mosselman, S., van Zoelen, E.J. and Olijve, W. (2000) Mol. Endocrinol. 14, 623–633.
[36] Morini, M. et al. (2000) Int. J. Cancer 87, 336–342.
[37] Kim, S.J. et al. (1997) Anticancer Res. 17, 2319–2323.
[38] Fujiwara, M., Jin, E., Ghazizadeh, M. and Kawanami, O. (2002) Pathol. Int. 52, 204–213.
[39] Rudroff, C. et al. (2002) Clin. Exp. Metastasis 19, 181–189.
[40] Kondo, S., Kubota, S., Shimo, T., Nishida, T., Yosimichi, G., Eguchi, T., Sugahara, T. and Takigawa, M. (2002) Carcinogenesis 23, 769–776.