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MDA-MB-453, an Androgen-responsive Human Breast Carcinoma Cell Line With High Level Androgen Receptor Expression

R.E. Hall, S.N. Birrell, W.D. Tilley and R.L. Sutherland

The role of androgens and the androgen receptor (AR) in the development and progression of breast cancer is poorly understood. To further define a potential model for androgen action in breast cancer, MDA-MB-453 cells, which express AR in the absence of oestrogen receptors and progesterone receptors, were further characterised in terms of AR expression and androgen responsiveness. High level expression of AR was confirmed by northern blot analysis, radioligand binding and immunocytochemistry, and could not be accounted for by AR gene amplification. Three endogenous androgen-responsive genes (fatty acid synthetase, gross cystic disease fluid protein of 15 kDa and prolactin receptor) and a transfected reporter gene, containing an androgen-responsive element, were induced following androgen administration. A synthetic androgen, mibolerone, induced moderate (27% above control) stimulation of MDA-MB-453 cell proliferation, which was abrogated by the simultaneous administration of the synthetic androgen antagonist, anandron, demonstrating that the effect was AR-mediated. In summary, MDA-MB-453 cells express high levels of functional AR, and thus provide a valuable *in vitro* model for further studies on androgen regulation of gene expression, and perhaps cell proliferation in breast cancer.

Key words: androgen action, breast cancer, androgen receptor
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INTRODUCTION

THERE IS ample justification for extending studies on androgen action mediated by the androgen receptor (AR) in human breast cancer. A greater proportion of breast cancer patients with AR-positive tumours respond to endocrine therapy and survive longer than patients with AR-negative tumours [1]. In addition, a recent reassessment of AR expression, using more sensitive techniques, indicated that up to 80% of breast cancer patients are AR+ and more significantly, that tumours may retain AR expression when oestrogen (ER) and progesterone receptor (PR) expression are undetectable [2]. Furthermore, the recent observation ascribing a germline mutation in AR as the probable cause of breast cancer in two brothers with androgen insensitivity [3], illustrates the requirement for more detailed studies on the role of androgens and AR in the development and progression of breast cancer.

Prior to the establishment of human breast cancer cell lines *in vitro*, Shionogi S115 mouse mammary tumour cells, which proliferate in response to androgens, were the primary model for androgen responsiveness in breast cancer [4]. Among the human breast cancer cell lines which have been established in culture, MDA-MB-453 cells stand out, by virtue of their high level of AR expression [5], as a potentially useful model for further study of androgen action in breast cancer.

MDA-MB-453 is a human breast carcinoma cell line with a modal chromosome number of 45, derived from a pleural effusion of a 48-year-old Caucasian woman with metastatic disease [6]. In culture, these cells grow as single cells or loosely

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attached groups of small round cells with a population doubling time of 2–3 days [7]; they do not form tumours in nude mice [6] or express the predominant features of an invasive phenotype [8]. The major distinguishing feature previously characterised is the amplification and overexpression of the proto-oncogene c-*erb*B-2 [9].

To date, there are few published data on the androgen responsiveness of these cells. Our preliminary characterisation of MDA-MB-453 cells indicated a high level of AR mRNA, which was confirmed by a radioligand binding assay [5]. In contrast, ER and PR were not detectable at the mRNA level in MDA-MB-453 cells. These observations, coupled with the recent finding that ER and PR mRNAs are often coexpressed with AR mRNA in other breast cancer cell lines [10], suggested that androgens may be key growth regulators in the MDA-MB-453 breast cancer cell line. This led us to further characterise these cells in terms of AR expression and androgen responsiveness.

Proliferative responses of human breast cancer cells to androgens have been reported to be either stimulatory or inhibitory [11–14]. The divergent effects of androgens on breast cancer cell lines may reflect cell-specific differences in the regulation of proliferation by androgens, and suitable model systems are necessary to further define these effects. Recently, it was reported that the growth of the breast cancer cell line, MFM-223, which also expresses a high level of AR, is significantly inhibited by concentrations of 5α-dihydrotestosterone (DHT) as low as 0.01 nM [13]. Accordingly, we have examined the effect of androgen on MDA-MB-453 cell proliferation and gene expression to further characterise AR functionality and androgen responsiveness in these cells.

MATERIALS AND METHODS

Materials

Cell culture reagents were obtained from CSL (Parkville, Victoria, Australia), Cytosystems (Castle Hill, NSW, Australia) and Flow Laboratories (Seven Hills, NSW, Australia), tissue culture plates were from Linbro (Sydney, NSW, Australia) and tissue culture flasks from Corning Glassware (Crown Scientific, Moorebank, NSW, Australia). Steroid hormones, DHT, 17β-oestradiol and 7α,17α-dimethyl-19-nortestosterone (mibolerone) were supplied by Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Tritium-labelled steroids, 5α-dihydro[1,2,4,5,6,7-^{3}H]testosterone (126 Ci/mmol), [2,4,6,7,16,17-^{3}H]oestradiol (140–158 Ci/mmol) and the synthetic progestin, 16α-ethyl-21-hydroxy-19-nor[6,7-^{3}H]pregn-4-ene-3,20-dione (Organon 2058) (47–52 Ci/mmol) and unlabelled Organon 2058 were supplied by Amersham (Sydney, NSW, Australia). RU 23908, 5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl]-2,4-imidazolinedione (anandron) was a kind gift from Dr J.P. Raynaud of Roussel-Uclaf (Romainville, France).

Cell culture

The human breast cancer cell lines MDA-MB-453 and ZR-75-1 were obtained from ATCC (Rockville, Maryland, U.S.A.); MCF-7 cells were provided by Dr C.M. McGrath, Meyer L. Prentis Cancer Center (Michigan, U.S.A.); T-47D and MDA-MB-231 cells were supplied by E.G. and G. Mason Research Institute (Worcester, Massachusetts, U.S.A.). The human prostate cancer cell line, LNCaP, was obtained from Dr J.S. Horoszewicz, Roswell Park Memorial Institute (Buffalo, New York, U.S.A.). Stock monolayer cultures were maintained in exponential growth, in RPMI 1640 medium supplemented with 6 mM L-glutamine, 10 μg/ml human insulin and 10% fetal calf serum (FCS), buffered with 14 mM sodium bicarbonate, 20 mM Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and containing 20 μg/ml gentamicin. Phenol red-free RPMI medium, used in growth studies, was supplemented as for stock cell culture with the following exceptions: the FCS was steroid-depleted by charcoal stripping (sFCS) [15] and the concentration of insulin reduced to either 1 μg/ml or not added to the medium. In the absence of added insulin, the rate of MDA-MB-453 cell proliferation was halved. All cell lines were free from *Mycoplasma* contamination (Gen-Probe Tissue Culture Rapid Detection System, BioMediq, Doncaster, Australia). DNA fingerprinting was employed to establish the authenticity of cell lines and ploidy was monitored by DNA flow cytometry.

Ligand binding assays

Exchangeable ligand binding was assayed in whole cells, growing as monolayers in 12-well tissue culture plates, by the method previously described [5, 16]. Cells were washed with binding buffer (serum-free, insulin-free and phenol red-free RPMI 1640 culture medium containing 0.1% w/v bovine serum albumin, radioimmunoassay grade, Sigma) then incubated in binding buffer containing [^{3}H]DHT, 17β-[^{3}H]oestradiol or [^{3}H]Organon 2058 (0.1–5 nM) for AR, ER and PR assays, respectively, ±100-fold molar excess of unlabelled steroid at 37°C for 1–1.5 h. Specifically bound radioactivity was measured after removal of unbound label by washing in ice-cold phosphate buffered saline (PBS) containing 5% w/v bovine serum albumin for 20 min on ice. Receptor binding, expressed as sites/cell, was determined from the molar concentration of ligand specifically bound and the number of cells per well. Soluble AR binding was assayed in high salt (0.4 M KCl) cell extracts, as reported previously [5].

Immunocytochemistry

A rabbit polyclonal antibody that recognises the carboxyl terminal (amino acids 898–917) of the human AR was used for immunocytochemical analysis [17]. After harvesting, cells were deposited on to poly-L-lysine (Sigma) coated slides by cytocentrifugation. The cells were fixed in buffered formalin, washed sequentially in methanol, acetone and PBS, then incubated for 15–30 min in blocking serum (3% goat plus 1% human sera in PBS). The primary AR antibody was diluted 1/500 and the control normal rabbit immunoglobulin was diluted 1/2000 in blocking serum. Incubation was carried out overnight at 4°C, followed by three washes in PBS to remove excess non-specifically bound immunoglobulin. The bound antibody was detected by a secondary, biotinylated goat anti-rabbit antibody, and after three washes, binding was visualised using a standard Vecta-Elite (streptavidin–biotin complex) staining kit (Vector Labs, Burlingame, California, U.S.A.), using diaminobenzidine tetrahydrochloride (DAB) as the chromogen ([17] and references therein). After immunostaining, the cells were counterstained for 30 s with weak haematoxylin (1:1 v/v Lillie Mayer's haematoxylin:distilled water), dehydrated and mounted for light microscopy. Positively stained cells were identified as brown and AR-negative cells were counterstained blue.

Quantitative analysis of AR immunocytochemistry

The area and integrated optical density (IOD) pixel density units of immunostaining were estimated using an automated image analysis system (Video Pro 32, Leading Edge Pty Ltd, Bedford Park, SA, Australia) calibrated for the purpose. In each

image field (200× magnification), the mean percentage of AR+ stained cells was calculated from the area of DAB stain/area of DAB + area of haematoxylin stain. The mean IOD is also an estimate of the amount of AR present in each image field. The mean optical density (OD) is a measure of the level of AR present in each cell. The number of fields measured (n = 20) was determined by the cumulative quotient principle to achieve a variance around the mean of ±5% [18].

Northern blot analysis

Northern blot analysis was performed using 20 μg of total RNA isolated from cell pellets using guanidinium isothiocyanate and caesium chloride gradient centrifugation as described previously [10]. The human AR cDNA was a 0.7 kb probe that encompasses most of the DNA binding domain and approximately half the hormone binding domain of the receptor [19]. A 2-kb *Eco*RI fragment of human fibroblast β-actin was provided by Dr P. Gunning (Children's Medical Research Institute, Westmead, NSW, Australia) [20]. A 2.3-kb PRLR cDNA, the H1/H2 probe covering the entire coding region, was provided by Dr P.A. Kelly (INSERM-U 344, Faculté de Médicine Necker-Enfants Malades, Paris, France) [21]. A 0.6-kb cDNA covering the coding region of a prolactin-inducible protein (PIP), which is identical to the gross cystic disease fluid protein of 15 kDa M_r (GCDFP-15), was provided by Dr L.C. Murphy (University of Manitoba, Winnipeg, Canada) [22]. A 2-kb cDNA for fatty acid synthetase (FAS) was provided by Dr H. Rochefort (INSERM-U 148, Unité d'Endocrinologie Cellulaire et Moléculaire, Montpellier, France) [23]. A 30 base pair oligonucleotide complementary to bases 151–180 of the rat 18S ribosomal RNA (rRNA) gene was used as a control for RNA quantitation [10].

Southern blot analysis

Genomic DNA was isolated from breast cancer cell lines and peripheral blood lymphocytes during RNA isolation. After caesium chloride centrifugation, the top 5 ml of the 10 ml guanidinium-isothiocyanate supernatant was discarded and the next 4 ml collected for DNA extraction. Six millilitres of water were added to dilute the 4 ml of supernatant, followed by standard phenol-chloroform extractions, ethanol precipitation and resuspension of the DNA in water [24]. DNA was digested with the restriction enzyme, *Eco*RI, and 10 μg DNA/cell line was subjected to electrophoresis on a 1% agarose gel. DNA M_r markers were run in adjacent lanes. Gels were washed in water and the DNA depurinated by soaking in two changes of 0.25 M HCl for 5 min. DNA was transferred to Zetaprobe membranes by capillary transfer in 0.4 M NaOH for 4 h. Filters were rinsed in 2× standard saline phosphate EDTA (SSPE) containing 0.1% sodium dodecylsulphate (SDS). Southern blots were probed with ^{32}P-labelled AR cDNA and re-probed with β-actin cDNA as a control, under the following conditions. Hybridisation was overnight at 65°C in 0.5 M NaH_2PO_4 pH 6.9, 5% Blotto, 0.1 M NaEDTA pH 8.0, 7% SDS. After hybridisation, the membranes were rinsed successively in 2× standard saline citrate (SSC); 2×SSC, 0.1% SDS and 0.05 × SSC, 0.1% SDS at room temperature and washed for 30 min at 65°C in 0.05×SSC, 0.1% SDS. The membranes were then exposed to X-ray film as for northern blot analysis.

Androgen-responsive gene expression

Cells were grown in 150-cm^3 flasks in phenol red-free RPMI 1640 medium supplemented with 10 μg/ml insulin and 5% FCS. Fresh medium with 5% sFCS was added 48 h prior to DHT (10 nM) or ethanol vehicle (control) addition. DHT was prepared as 1000-fold concentrated stock solutions in ethanol and added to the medium to a final concentration of 0.1%. RNA was isolated as described above and northern blots were hybridised as described previously [10], using radioactively labelled cDNA probes.

MDA-MB-453 cells were cultured in DMEM medium and transiently transfected by calcium phosphate precipitation as described previously [5]. The plasmids, pMSGCAT, containing an androgen-response element within the mouse mammary tumour virus long terminal repeat (MMTV LTR), and pCH110, containing bacterial β-galactosidase sequences (a control to monitor transfection efficiency), were obtained from Pharmacia LKB Biotechnology (North Ryde, NSW, Australia). After transfection, the cells were washed with fresh medium before the addition of 10 nM DHT. Cells were harvested at 24 and 48 h after DHT addition. Cells were lysed by freeze-thawing (3×) and aliquots of the supernatant taken to measure protein concentration (Micro BCA reagents, Pierce, Rockford, Illinois, U.S.A.), β-galactosidase activity measured spectrophotometrically and chloramphenicol acetyltransferase (CAT) activity as described previously [5].

Growth studies

MDA-MB-453 cells were plated in 96-well plates at 3×10^4 cells/well in 100 μl RPMI 1640 medium containing 10% FCS and 1 μg/ml insulin or 10% sFCS without insulin. Cells were allowed to undergo two to three population doublings prior to the addition of vehicle or mibolerone (10 nM) ± the androgen antagonist, anandron (0.1 μM). Media and steroids were replenished every 3 days and cells were subconfluent at harvest. Cell number was determined by a colorimetric assay: cells were fixed by the addition of 10% (v/v) formaldehyde in normal saline for 30 min prior to staining with 1% (w/v) methylene blue in 0.01 M borate buffer (pH 8.5) [25]. Excess dye was removed by four successive washes in 0.01 M borate buffer and bound dye was eluted in a 1:1 (v/v) ethanol and 0.1 M HCl solution. The absorbance of the eluate was determined at 650 nm using a plate-reading spectrophotometer (Bio-Rad, Australia). Cell number was read from a standard curve.

RESULTS

We have previously reported that MDA-MB-453 cells are unusual among breast cancer cell lines in expressing a high level of AR mRNA without detectable ER or PR mRNA [5]. This result was confirmed in the present study where long-term exposure of autoradiograms failed to detect any ER or PR mRNA transcripts (data not shown). These studies were extended to determine if there were comparable increases in AR protein and AR binding. Whole cell radioligand binding assays revealed an approximate 4-fold increase in AR binding activity when compared with T-47D cells, which express AR at a level similar to the majority of AR+ breast cancer cell lines ([5] and Table 1). Compared with other AR+ human breast cancer cell lines, MDA-MB-453 cells also showed intense staining for AR by immunocytochemistry, whereas in MDA-MB-231 AR-negative breast cancer cells, nuclear AR staining was virtually absent (Figure 1). Quantitative comparisons with two other AR+ breast cancer cell lines, T-47D and MCF-7, and the human prostate cancer cell line, LNCaP, which is known to express very high levels of AR [5, 26], confirmed the high level expression of AR protein in MDA-MB-453 cells (Table 2). The percentage of AR-positive cells in MDA-MB-453 cultures was comparable to

Table 1. Androgen, oestrogen and progesterone receptor concentrations determined by ligand binding assays: a comparison of MDA-MB-453 and T-47D cells

Cell line	Receptor sites/cell AR	ER	PR
MDA-MB-453	73690 ± 7235	0	0
T-47D	16000	21000	1090000

AR, androgen receptor; ER, oestrogen receptor; PR, progesterone receptor. Receptor concentrations were measured by whole cell radioligand binding assays. Mean ± S.E. of AR concentration in MDA-MB-453 cells ($n = 6$).

Table 2. Quantitation of androgen receptor immunocytochemistry by video image analysis

Cell line	Percentage AR+	Optical density (OD)	Integrated optical density (IOD)
MDA-MB-453	85.9 ± 2.8	0.45 ± 0.01	14988 ± 946
T-47D	32.4 ± 5.4	0.28 ± 0.00	4186 ± 875
MCF-7	20.5 ± 4.8	0.24 ± 0.02	2674 ± 861
LNCaP	90.9 ± 4.2	0.46 ± 0.01	22298 ± 2242

The mean ± S.E. % AR+ stained cells, OD and IOD for MDA-MB-453, T-47D and MCF-7 breast cancer cells and LNCaP prostate cancer cells (depicted in Figure 1), calculated from the mean number of cells measured in at least 20 image fields/cell line in order to analyse a similar number of nuclei/cell line.

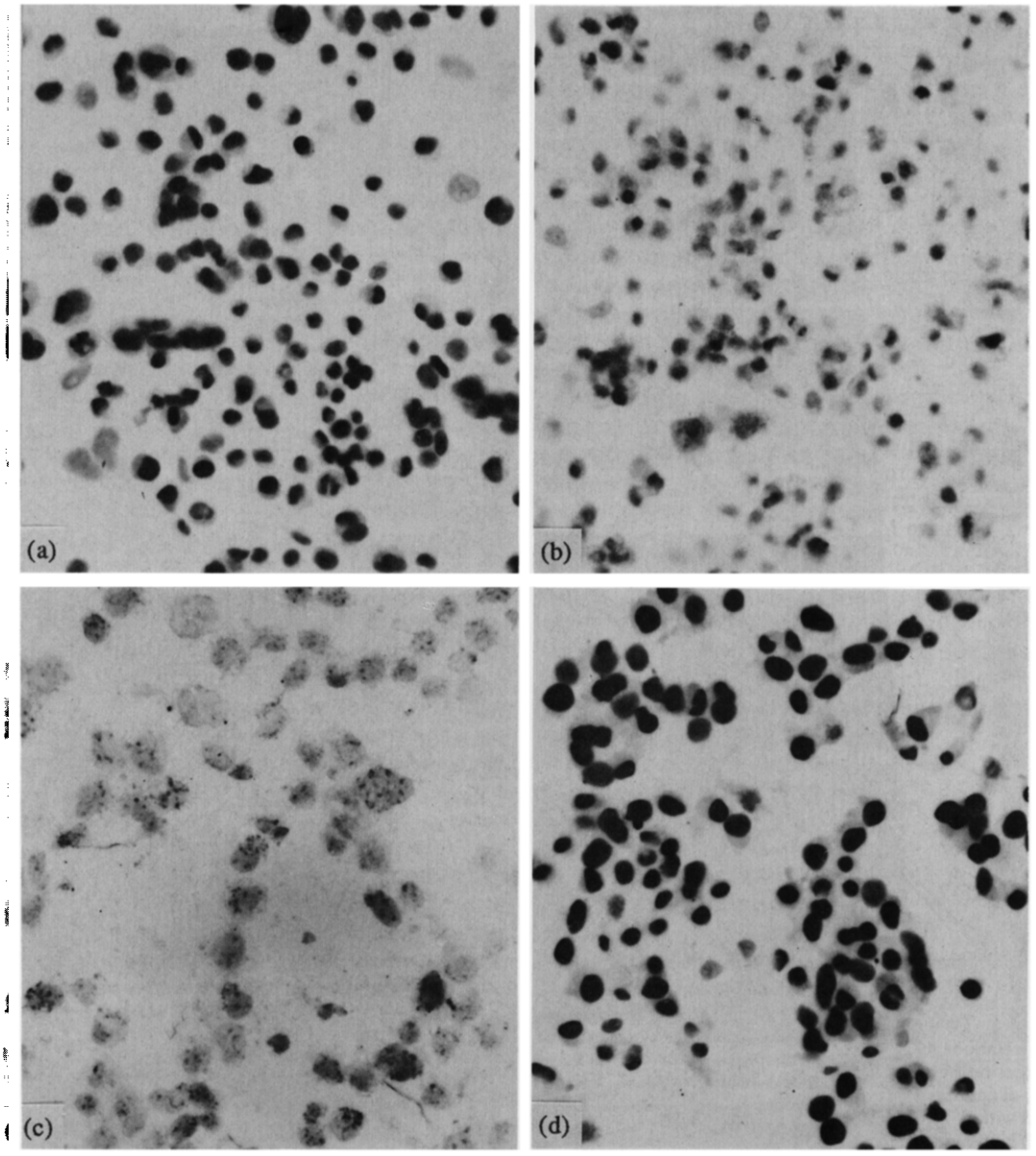

Figure 1. Androgen receptor immunocytochemistry in human breast cancer cells and LNCaP prostate cancer cells. (a) MDA-MB-453, (b) T-47D (AR+) and (c) MDA-MB-231 (AR− control) human breast cancer cells and (d) LNCaP prostate cancer cells. Original magnification 250×. Scale: 1 cm = 40 μm.

that seen with LNCaP cells, i.e. 85–90%, which was 3–4-fold greater than in MCF-7 and T-47D cells.

The mean IOD obtained from the image analysis of AR immunocytochemistry concurred with other measures of AR expression, i.e. mRNA levels and ligand binding assays, in MCF-7, T-47D, MDA-MB-453 and LNCaP cells (Figure 2). The IOD of AR immunostaining was positively correlated with both the relative level of AR mRNA determined by northern blot analysis ($r = 0.99, P = 0.01$) and with the total soluble AR concentration determined by ligand binding assay ($r = 0.99$, $P = 0.01$). Regardless of the parameter used to measure AR expression, the same relative hierarchy was observed between LNCaP prostate cancer cells, MDA-MB-453 and the other breast cancer cell lines, i.e. LNCaP > MDA-MB-453 > T-47D > MCF-7, and AR was not detectable in MDA-MB-231 cells.

To determine whether the high level of AR mRNA and protein in MDA-MB-453 cells is due to amplification of the AR gene, Southern blot analysis compared AR gene copy number in MDA-MB-453 cells with that of T-47D and ZR-75-1 breast cancer cell lines and normal peripheral blood lymphocytes. There was no evidence of gross rearrangements or amplification of the AR gene in MDA-MB-453 cells; β-actin served as a control for DNA loading and transfer (Figure 3). There was also no evidence of gross aneuploidy in this cell line; the DNA index of MDA-MB-453 cells was 2.2, compared with a normal diploid index of 2.0, as determined by analytical flow cytometry.

To test that the AR expressed by MDA-MB-453 cells is functional, cells were treated with a receptor-saturating concentration of DHT, i.e. 10 nM. We have shown previously that AR mRNA is downregulated by its own ligand [5] and this was confirmed here (Figure 4), along with a demonstration of induction of three known androgen-responsive genes. The levels of mRNA for all three genes (FAS, GCDFP-15 and PRLR) were induced to maxima ranging from 120 to 170% of control (Figure 4a and b). Androgen-regulated gene expression was further investigated in a transient transfection system employing the androgen-responsive MMTV LTR linked to the reporter gene, CAT. As shown in Figure 4c, CAT activity increased linearly to a maximum 7-fold induction over the 48-h study period. These data demonstrate that the AR is functional in MDA-MB-453 cells.

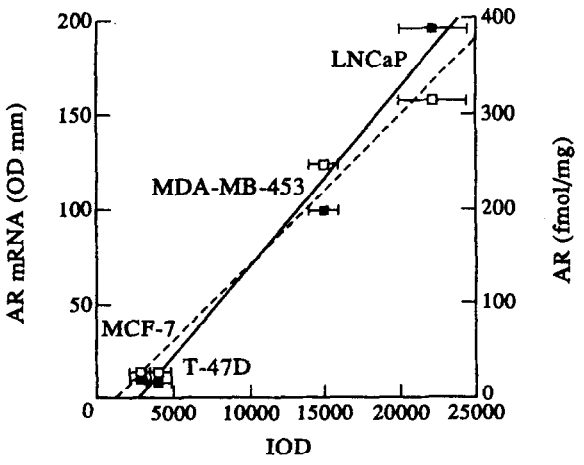

Figure 2. Correlations between different parameters of androgen receptor expression in breast and prostate cells. Integrated optical density (IOD) of androgen receptor (AR) immunocytochemical staining correlated with AR mRNA (■, solid line) and 5α-dihydrotestosterone (DHT) binding (□, dashed line) in MDA-MB-453, T-47D and MCF-7 breast cancer cells and LNCaP prostate cancer cells. The lines of best fit were determined by linear regression analysis and the correlation coefficients ($r = 0.99$) were the same in both cases, with highly significant associated probability values ($P = 0.01$).

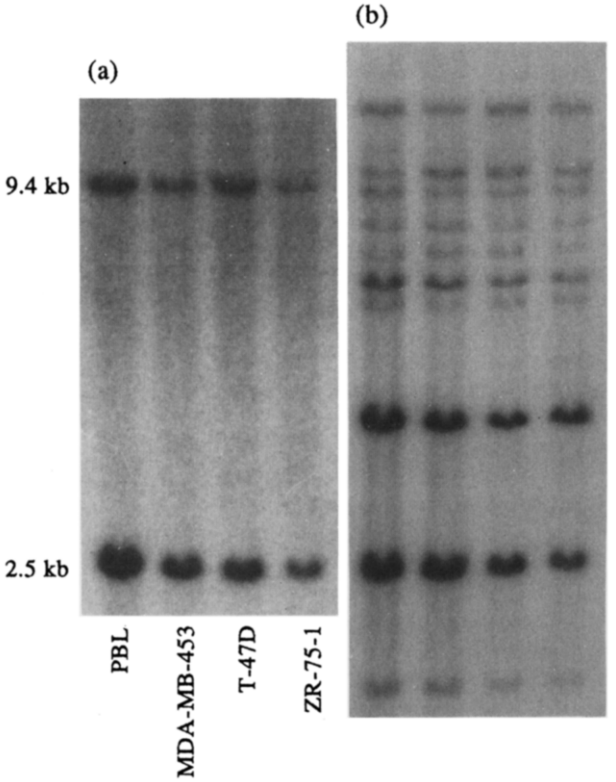

Figure 3. Southern blot analysis of androgen receptor (AR) gene copy number. (a) 10 μg genomic DNA from three human breast cancer cell lines and peripheral blood leucocytes (PBL) from a normal female donor, digested with *Eco*RI and probed with labelled AR cDNA. The autoradiogram was exposed for 6 days with an intensifying screen. (b) The same Southern blot probed with β-actin cDNA and exposed for 16 h.

Finally, the effects of the synthetic androgen, mibolerone, on the proliferation of MDA-MB-453 cells was investigated in medium known to give near maximal proliferation rates (10% FCS + insulin) and in a steroid and growth factor depleted medium (10% sFCS without insulin). These experimental conditions were chosen to maximise the likelihood of observing inhibitory and stimulatory effects, respectively. When MDA-MB-453 cells were cultured in medium containing 10% sFCS without supplemental insulin, the population doubling time of untreated cells was approximately doubled (from 2.5–3 days to 6 days), and under these conditions, proliferation was stimulated to 27% above control levels by 10 nM mibolerone (Figure 5). Simultaneous addition of the androgen antagonist, anandron, abrogated the stimulatory effect of 10 nM mibolerone (Figure 5), indicating that the modest growth stimulatory effect was AR mediated. An even more modest stimulatory effect of mibolerone was observed in 10% FCS. More importantly, there was no evidence of androgen-induced growth inhibition in these experiments.

DISCUSSION

These data confirmed an earlier observation that MDA-MB-453 cells stand out from other breast cancer cell lines because they express high levels of AR mRNA in the absence of ER and PR [5, 10]. More detailed analysis of protein expression by

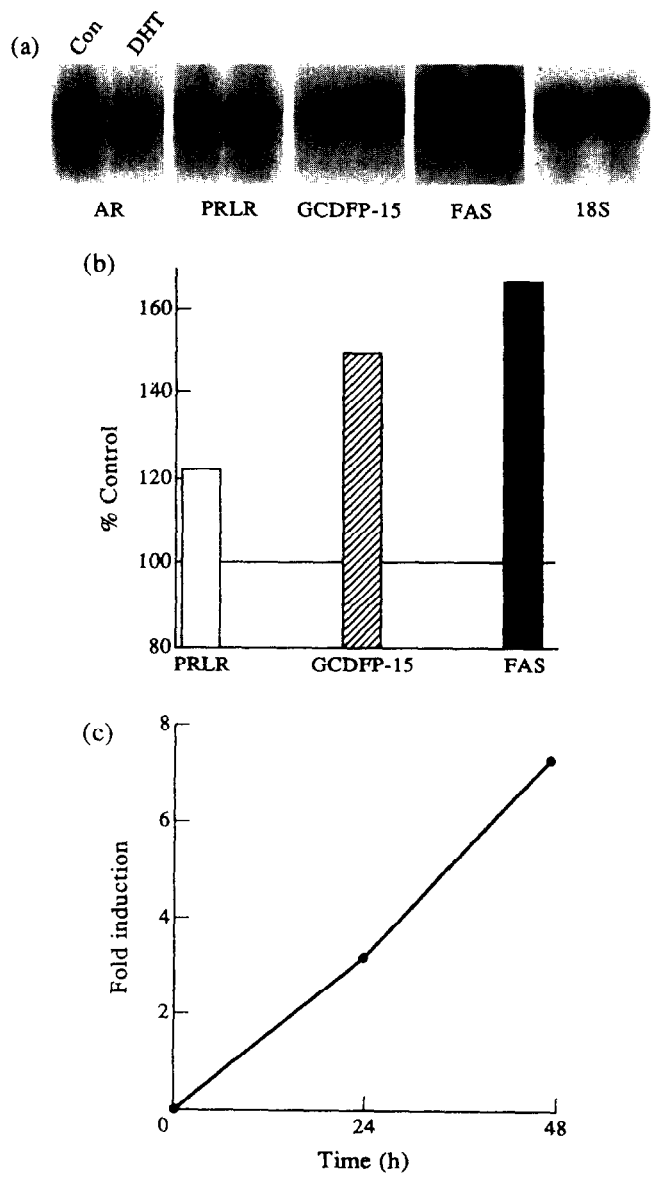

Figure 4. Induction of androgen-responsive genes in MDA-MB-453 cells. (a) Northern blots of MDA-MB-453 cells after 48 h treatment with 10 nM 5-α-dihydrotestosterone (DHT) or vehicle (con, control). Autoradiograms for androgen receptor (AR) (10.5 kb mRNA) and PRLR (13.5 kb) were exposed for 2 days, GCDFP-15 (0.9 kb) for 5 h, FAS (8 kb) for 16 h and control 18S rRNA (1.9 kb) for 7 min, respectively. (b) PRLR, GCDFP-15 and FAS mRNA levels were measured by densitometry and data are expressed as per cent induction relative to controls. (c) Time course of CAT reporter gene activity (●), following 10 nM DHT treatment of MDA-MB-453 cells transiently transfected with MMTV LTR-CAT. Fold induction of CAT activity is expressed relative to untreated transfected controls. Error bars representing the standard error of the mean of triplicate determinations did not exceed the size of the symbol.

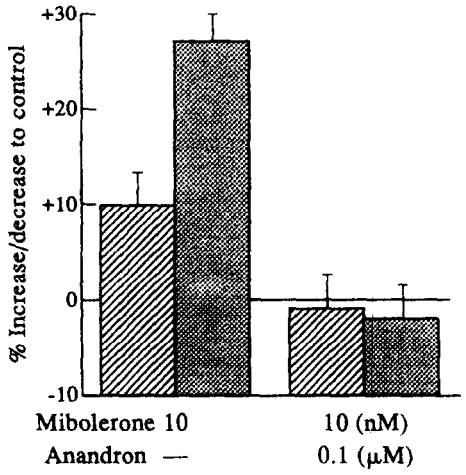

Figure 5. Growth effects of mibolerone in MDA-MB-453 cells. MDA-MB-453 cells were cultured in either medium containing 10% fetal calf serum (FCS) with 1 μg/ml insulin (▨) or medium containing 10% sFCS without insulin (▩). Cells were harvested 15 days after treatment and are expressed relative to vehicle-treated controls which were grown in the same medium. Error bars represent the standard error of the mean of 12 wells per treatment.

immunocytochemistry and steroid binding analysis confirmed the absence of ER and PR in these cells, and demonstrated that the high levels of AR mRNA are translated into high levels of functional AR protein, as assessed by its ability to bind androgens with appropriate affinity, and to induce the expression of known androgen-responsive genes. We have shown previously that androgens acting via the AR increase PRLR binding in MDA-MB-453 and MCF-7 breast cancer cells [21].

In our current study, DHT induction of a transfected gene containing an androgen-response element within the MMTV LTR linked to CAT further demonstrated the functionality of AR in MDA-MB-453 cells, while the induction of three endogenous genes demonstrated that the cells are androgen-responsive. Androgens may promote or help maintain a more differentiated breast tumour phenotype, which is generally associated with a better patient prognosis. In addition to MDA-MB-453 cells, DHT induced GCDFP-15 and FAS mRNAs in T-47D cells (data not shown). It has been shown previously that the induction of FAS, in response to either progestin or androgen, caused lipid accumulation in T-47D breast cancer cells [27]. GCDFP-15 has been detected in the blood of some breast cancer patients [28], and induced by DHT in T-47D [22, 29] and ZR-75-1 breast cancer cells [30]. Additionally, both FAS and GCDFP-15 are characteristic of apocrine gland secretions.

The steroid hormone receptor profile of MDA-MB-453 cells reported here is very similar to that of the recently derived MFM-223 human breast cancer cell line, which also expresses high AR, without ER or PR [13, 31]. Since it has been suggested that AR is the last of the sex steroid hormone receptors to be lost during progression from a receptor-positive phenotype to a receptor-negative phenotype [2], these two cell lines may provide examples of a similar phenotype in this progression.

A major difference between these two apparently similar cell lines is their proliferative reponses to androgens. MDA-MB-453 cells showed a modest stimulation of growth which was greater in minimal medium. In contrast, MFM-223 cells were markedly growth-inhibited by androgen [13]. Thus, while MDA-MB-453 and MFM-223 cells have a similar receptor profile, the proliferative response of each cell line differs appreciably. ZR-75-1 cells are also growth inhibited by androgens [12], while proliferation of MCF-7, EFM-19 [11] and EVSA-T breast cancer cells [14] is stimulated. These diverse responses illustrate the need for a more detailed understanding of the mechanisms of androgenic growth control in human breast cancer.

Variations in androgen metabolism may contribute to the divergent effects of androgens on the *in vitro* growth of human breast cancer cells, as stimulatory effects may be mediated by aromatisation of androgens to oestrogens. However, MDA-MB-453 cells are ER−, and the antagonism of androgen-induced

growth stimulation by anandron clearly indicates an effect mediated by AR. It will be interesting to test whether these cells are androgen-dependent for growth in nude mice in view of their inability to grow in untreated animals [6].

The proliferative response of individual breast cancer cell lines to androgen is not directly related to the autologous regulation of AR. In MDA-MB-453 and EFM-19 cells, androgen downregulates AR and stimulates proliferation (Figures 4 and 5 [5, 32]). In MFM-223 cells, androgen downregulates AR, but inhibits proliferation [32], while in EVSA-T cells, androgen upregulates AR and stimulates proliferation [14]. Thus, there are cell-specific differences in both androgen regulation of AR expression and androgen regulation of growth, even where the culture conditions for different cell lines are identical.

Presumably, the androgen regulation of breast cancer cell proliferation is as complex as that for oestrogens, involving multiple interactions with other hormones and growth factors present in the growth medium or produced by the cells themselves [33]. This complexity necessitates future detailed study of the molecular basis for androgen regulation of breast cancer cell proliferation, and the MDA-MB-453 and MFM-223 cell lines provide potentially useful *in vitro* models with which to elucidate these mechanisms.

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