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REGULATION OF ANDROGEN RECEPTOR mRNA AND PROTEIN LEVEL BY STEROID HORMONES IN HUMAN MAMMARY CANCER CELLS

REINHARD HACKENBERG,[1]* THOMAS HAWIGHORST,[1] ANGELIKA FILMER,[1] EMILY P. SLATER,[2] KARIN BOCK,[1] MIGUEL BEATO[2] and KLAUS-DIETER SCHULZ[1]

[1]Zentrum für Frauenheilkunde und Geburtshilfe, Philipps Universität, Pilgrimstein 3 and [2]Institut für Molekularbiologie und Tumorforschung, Philipps Universität, Emil-Mannkopffstaße 2, W-3550 Marburg, Germany

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Summary—The regulation of the human androgen receptor (AR) by steroid hormones in human mammary cancer cells was investigated using immunocytochemical and ligand binding assays for its protein and Northern blot analyses for the corresponding mRNA. MFM-223 cells contain high levels of ARs and are growth-inhibited by dihydrotestosterone (DHT). The AR protein is down-regulated to 57% of the control by 10 nM DHT after 24 h, and the corresponding mRNA is also reduced. The nonsteroidal antiandrogen hydroxyflutamide had no effect on the AR level, whereas after incubation with 1 μM cyproterone acetate a slight down-regulation was observed. The AR level was restored completely after release from a 7 day treatment with DHT. However, only 60% of the control level was restored, if the cells were grown in the presence of DHT for 6 weeks. In androgen-pretreated cells the proliferation rate remained decreased even after the withdrawal of DHT. Concomitantly the distinct growth inhibition was lost. Transfection experiments demonstrated a reduced activity of the residual androgen receptor in these pretreated cells.

In addition to the AR, EFM-19 cells also contain significant amounts of estrogen and progesterone receptors. EFM-19 cells are not growth inhibited by physiological concentrations of DHT. Autoregulation of AR was also found in this cell line. Additionally, reduced levels of AR protein and mRNA were found in EFM-19 cells after treatment with the synthetic progestin R5020. The maximum effect of R5020 was observed at the high concentration of 1 μM. Estrogen treatment with 10 nM 17β-estradiol for 3 days reduced the AR level only by 25%.

INTRODUCTION

As shown by many investigators, receptors for different steroid hormones are frequently detectable in human breast cancer tissue as well as in cell lines derived from this type of tumor. The detection of estrogen and progesterone receptors allows relatively accurate identification of hormone-responsive tumors. The probability of response to different endocrine treatment regimens is between 75 and 85%, if the individual breast tumor is positive for both receptors [1]. While the prognostic value of the estrogen receptor remains controversial, the presence of progesterone receptors in the tumor correlates with an increased, disease-free survival [2].

Androgen receptors (ARs) above 20 fmol/mg protein were found in 30 to 50% of human breast cancer specimens investigated [3–7]. Using an improved method, a cytosolic concentration of ARs above 10 fmol/mg protein was detected in 85% of the 852 primary tumors tested [8]. ARs are found preferentially in combination with estrogen or progesterone receptors, and only less than 10% of the tumors contain only ARs [7, 8]. In estrogen and progesterone receptor-positive tumors the additional presence of ARs helps to identify breast cancer patients with an improved prognosis [9].

Yet there is only limited knowledge on the functional role of androgens and their receptors in the development and progression of breast cancer. Androgenic compounds such as testosterone propionate have been successfully used in the treatment of disseminated breast cancer [10].

*To whom reprint requests should be addressed.

Abbreviations: AR, androgen receptor; CAT, chloramphenicol acetyltransferase; DHT, 5α-dihydrotestosterone; DCM, culture medium with dextran-coated charcoal-treated serum; E_2, 17β-estradiol; GM, standard growth medium; MMTV, mouse mammary tumor virus; R1881, methyltrienolone; R5020, promegestone; RSV, Rous sarcoma virus.

However, this type of hormonal therapy was replaced later by other endocrine treatment modalities, showing a higher efficiency and less side effects. The clinical observations corroborate the possible significance of androgens as regulatory factors in human breast cancer.

In vitro ARs were found in the permanent human breast cancer cell lines MCF-7 [11], T-47D [12], ZR-75-1 [13], EFM-19 and MFM-223 [14]. The proliferation of ZR-75-1 [15] and MFM-223 cells [14] was already inhibited by low androgen concentrations, e.g. 1 nM dihydrotestosterone (DHT), whereas MCF-7 [16] and EFM-19 cells [17] were only stimulated by high concentrations of DHT (1 μM). T-47D cells were not responsive to DHT [18]. Steroid receptors are not only passive links in the cascade of steroidal action, but they are also targets of hormonal regulation by different steroids including their own ligands. Alterations in the steroid receptor levels may modify the responsiveness of breast cancer cells to steroidal hormones. In MCF-7 cells AR protein level was reduced after treatment with estrogen [19] or progestin [20]. We have now investigated the effects of steroid hormones on AR protein and its mRNA in the human breast cancer cell lines MFM-223 and EFM-19.

MATERIALS AND METHODS

Cell lines and proliferation assays

MFM-223 [14] and EFM-19 [21] cells were grown in Eagle's minimal essential medium supplemented with 67 mg/l gentamicin sulfate (Biochrom, Berlin, Germany), 2.5 mg/l transferrin (Serva, Heidelberg, Germany), 40 IU/l insulin (Hoechst, Frankfurt, Germany) and 10% fetal bovine serum (Boehringer-Mannheim, Germany). Reduced medium (DCM), used in some of the experiments, was prepared free of transferrin, insulin and phenol red. Fetal bovine serum was treated with dextran coated-charcoal to eliminate endogenous steroids [22].

Proliferation assays were performed in multidishes (2 cm^2, Nunc, Roskilde, Denmark). After the indicated incubation periods the cells were detached from the wells by trypsinization and counted in a hemocytometer [17]. Androgen resistance was induced in separately cultivated MFM-223 cells. These cells were grown for 16 weeks in standard culture medium supplemented with 10 nM DHT.

Northern blot hybridization

Cells were lysed directly on the plate in 4 M guanidine isothiocyanate buffer. RNA was isolated by ultracentrifugation through a cesium chloride gradient. Poly(A)$^+$RNA was prepared from total RNA using oligo(dT)-cellulose columns ("Quick Prep", Pharmacia-LKB, Freiburg, Germany). 20 μg of total RNA or 10 μg of Poly(A)$^+$RNA was loaded onto 1.2% agarose–formaldehyde gels, run for 3–4 h at 80 V, transferred to nylon membranes (Biodyne A, Pall, Frankfurt, Germany), and fixed by baking for 2 h at 80°C [23]. The filters were hybridized with the ^{32}P-labeled 0.7 kb *HinD*III/*Eco*RI fragment of human AR cDNA (ARHLL1) [24]. Rehybridization was performed with β-actin and GADPH cDNAs to verify that equal amounts of RNA had been loaded.

AR binding assays

Cells were seeded in 4-well dishes and grown until near-confluence. The cultures were rinsed twice with PBS and supplied with 0.5 ml DCM per well. If the cell cultures were pretreated with unlabeled DHT, an extended washing protocol was applied. The cell layers were rinsed three times with GM and incubated for 1 h at 37°C. The fourth washing cycle was followed by the incubation with the labeled ligand. [^{3}H]R1881 (NEN, Frankfurt-Dreieichenhain, Germany) was added to duplicate samples in five concentrations ranging from 25 pM to 2.5 nM. Binding to the glucocorticoid receptor was prevented by the use of 3.5 μM triamcinonole acetonide (Sigma, Munich, Germany). Unspecific binding was estimated in parallel samples in the presence of 500 nM unlabeled R1881. After an incubation period of 4 h at 37°C, the cultures were rinsed twice with PBS followed by 0.25 ml 1 N NaOH to lyse the cellular material. After neutralization with 1 N HCl, aliquots were counted in a β-scintillation counter. The data were analyzed by Scatchard plots.

Transfection

One day prior to transfection, cells were plated out at a concentration of 0.5×10^6 cells per 60-mm cell culture dish. A mixture of supercoiled DNA including 4 μg CAT construct, either MMTV CAT [25] or RSV CAT [26] and 2 μg RSV β-galactosidase (βGAL) [27] was introduced to each plate by the calcium phosphate method [28]. 20 h posttransfection, media were

changed to DCM containing 5% fetal bovine serum and DHT was added. After 48 h the cells were collected and lysed by 3 cycles of freezing and thawing in 100 μl 0.25 M Tris–HCl, pH 7.8. Cytosolic extracts were tested for CAT [26] and βGAL [27] activity. The βGAL assay demonstrated the uniformity of transfection efficiency (data not shown).

Immunocytochemistry

Cells were grown on standard microscope slides in Petri dishes to subconfluence, fixed in 4% buffered formaldehyde for 10 min followed by cold methanol and acetone, each for 3 min. The monoclonal antibody F39.4.1 directed against the human AR [29] was kindly provided by Dr N. Zegers (Rijswijk, The Netherlands). The primary antibody was allowed to incubate overnight at 4°C in a humidified chamber [30]. Subsequently a universal kit for an indirect immunoperoxidase staining method was used (Amersham, Braunschweig, Germany). In the negative controls the primary antibody was omitted.

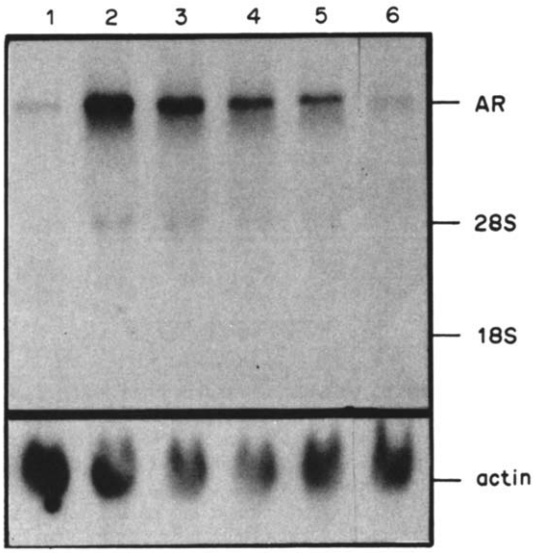

Fig. 1. Relative abundance of AR mRNA in MFM-223 (lane 2) in comparison with MCF-7 (lane 6) mammary cancer cells analyzed by Northern blot. Down-regulation of AR mRNA was demonstrated in MFM-223 cells after incubation with 10 nM DHT for various periods of time (lanes 3, 4 and 5: 6 h, 2 and 4 days, respectively; and 25 days in lane 1). The blots were rehybridized with a β-actin cDNA to verify the integrity and amount of total RNA loaded.

RESULTS

Regulation of AR mRNA in MFM-223 cells

MFM-223 cells are more abundant in AR mRNA than MCF-7 mammary carcinoma cells (Fig. 1). These data are in accordance with ligand binding assays demonstrating a 4-fold excess of AR protein in MFM-223 cells [14]. Androgen-induced down-regulation of AR mRNA in MFM-223 cells was apparent in Northern blot analyses. A reduction of mRNA was seen after 6 h and was more pronounced after prolonged incubation periods of up to 25 days. 20 μg RNA was loaded in each lane and this was verified by rehybridization with β-actin cDNA. The AR mRNA levels, evaluated relatively to the β-actin mRNA contents, were 91, 86, 33, and 7% after 6 h, 2, 4, and 25 days of incubation with DHT, respectively.

Binding assays of AR protein in MFM-223 cells

After preincubation with androgen, the unlabeled androgen must be removed prior to the incubation with [^{3}H]R1881. The efficacy of the washing protocol was verified after incubating MFM-223 cells with 10 nM DHT for 2 h at 4 and 37°C [Fig. 2(A)]. The Scatchard analyses revealed the following K_d-values: 0.074 nM (control), 0.086 nM (4°C), and 0.093 nM (37°C). The slight reduction of the affinity after preincubation with DHT was probably due to traces of DHT, that had not been removed. The binding capacity was $\geqslant$95% of the control at either temperatures.

After treating MFM-223 cells with 10 nM DHT for 24 h, the binding affinity of the AR remained constant, whereas the binding capacity was reduced to 57% of the control [Fig. 2(B)]. 1 μM DHT was equally effective with regard to the number of binding sites. The increase in the K_d-value (control: 0.055 nM) was slight after incubation with 10 nM (0.074 nM)

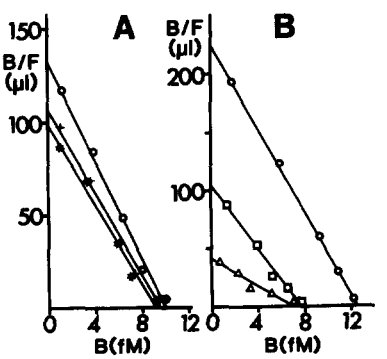

Fig. 2. Scatchard analyses of AR protein in MFM-223 cells after incubation with DHT. (A) After incubation with 10 nM DHT at 37°C (stars) or 4°C (crosses) for 2 h the cells were washed and compared with the control (circles). (B) AR protein was down-regulated after treating the cells with 10 nM (squares) or 1 μM (triangles) DHT for 24 h and compared with the control (circles).

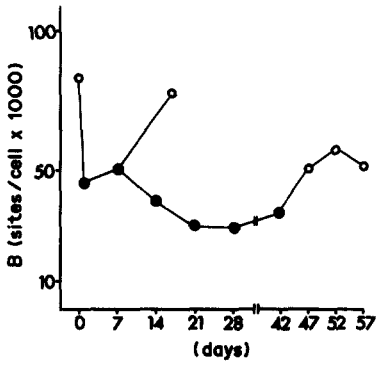

Fig. 3. Kinetics of AR autoregulation by DHT in MFM-223 cells. Down-regulation of AR during incubation with 10 nM DHT for up to 28 days (solid circles). Cells, which were released from DHT after 7 days, reached nearly the control level 10 days later (open circle). After long term incubation with DHT for 42 days (solid circle), only partial restoration of AR was observed after release from DHT (open circles).

and distinct after treatment with 1 μM DHT (0.174 nM). The increased K_d-value after the application of the high concentration of 1 μM DHT indicates incomplete removal of the unlabeled DHT applied before the assay incubation. The increased K_d-value was used to calculate the concentration of DHT in the incubation medium by an approach for competitive binding [17]. 0.1 nM DHT (0.01% of the initial concentration) could cause the observed increase of the K_d-value.

Treatment with androgen for up to 28 days was monitored by multiple Scatchard analyses. The AR levels decreased to 30–40% of the control after 14 days, and were nearly constant during further prolongation of the incubation periods. In additional experiments, MFM-223 cells were withdrawn from the androgen containing medium after 7 days, followed by the incubation under androgen-free conditions. The AR level increased again and nearly reached that of the control cells 10 days later (Fig. 3). After release from long term treatment with DHT for 6 weeks, the AR level was not restored completely and stayed constantly at 50–60% of the wildtype level (Fig. 3).

The influence of antiandrogens was compared with the effects of DHT. 1 μM of the nonsteroidal antiandrogen hydroxyflutamide had no effect on the AR level after an incubation period of 3 days. After application of 1 μM of the steroidal antiandrogenic compound cyproterone acetate, AR levels were reduced to 82% of the control, but this difference was not significant (Table 1). The constant K_d-values demonstrate that the binding of both antiandrogens to the AR remains in a completely exchangeable form.

Proliferation of MFM-223 cells

The incubation with 10 nM DHT significantly decreased MFM-223 cell growth. Cell numbers were 45% of the control after an incubation period of 15 days. MFM-223 cells preincubated with 10 nM DHT for 16 weeks, were released from androgenic inhibition. After withdrawal of DHT, the proliferation rate of these pretreated cells remained markedly reduced. The cell numbers were only 51% of wildtype MFM-223 cells, cultivated in parallel without androgen. During the continued incubation of the pretreated cells with 10 nM DHT the growth rate did not react significantly to the androgen application (Fig. 4). The mammary cancer cells seem to loose androgen responsiveness under the experimental conditions used.

Transfection

After introducing a DNA construction of the steroid hormone dependent MMTV promotor linked to the CAT gene into MFM-223 cells, the binding of the androgen–AR complex to the MMTV promotor induces the expression of CAT activity. No significant CAT activity was observed in the absence of DHT (Fig. 5). After incubation with 1 nM up to 1 μM DHT how-

Table 1. AR content and counts of MFM-223 cells after incubation with 1 μM of the antiandrogens cyproterone acetate (CPA), hydroxyflutamide (HF) or 10 nM DHT for 3 days in DCM

Cellular properties	Hormonal agent under investigation		
	1 μM CPA	1 μM HF	10 nM DHT
AR (sites/cell)	119,000 ± 19,000	145,000 ± 23,000	62,000 ± 15,000
(% of control)	82	99	42
K_d (nM)	0.11 ± 0.03	0.04 ± 0.01	0.07 ± 0.03
Cell counts (% of control)	86 ± 15	90 ± 8	77 ± 7

Values are expressed as means and standard deviations derived from 3 independent experiments. The control cells contained 145,000 ± 11,000 binding sites per cell with a K_d value of 0.04 ± 0.01 nM.

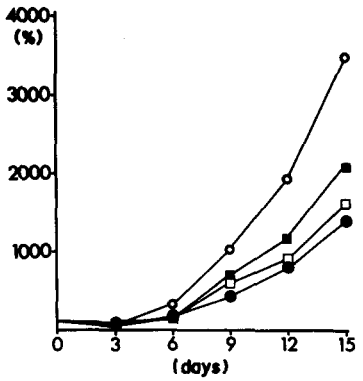

Fig. 4. Kinetics of cell proliferation of MFM-223 cells in standard culture medium. Proliferation of wild-type cells (open circles) was distinctly inhibited by 10 nM DHT (solid circles). After preincubation with DHT for 16 weeks and subsequent cultivation in standard culture medium, the growth rate of MFM–DHT cells was decreased (open squares). Adding DHT continuously, no androgen-induced inhibition of the proliferation of MFM–DHT cells was observed (solid squares). The slight stimulation of proliferation was not significant. All values were derived from quadruplicate cultures and expressed as percentages of the initial cell counts. Standard deviations were <10%.

ever, a high level of CAT activity was seen, demonstrating the functional activity of the AR. If compared with untreated wildtype MFM-223 cells, the transfection efficacy was lower in MFM-223 cells, pretreated with DHT for 17 weeks (MFM–DHT). But the reduced transfection rate was sufficient to evaluate the functional activity of the AR in these cells. Adding different concentrations of DHT to the androgen pretreated and transfected MFM-223 cells (MFM–DHT), only minimal CAT activity was found. This observation is not due to the complete loss of ARs. The analysis of the binding capacity in MFM–DHT cells demonstrated 27,000 binding sites per cell, corresponding well to other experiments (compare Fig. 3). These data demonstrate the loss of functional activity of the AR after long term pretreatment with DHT.

Down-regulation of the AR in EFM-19 cells by androgen, estrogen, and progestin

EFM-19 cells are another human mammary cancer cell line expressing ARs. In contrast to MFM-223 cells however, the growth rate of EFM-19 cells is not inhibited by androgen, but stimulated in the presence of DHT in the range of 0.1 to 1 μM [17]. The effect of DHT on the AR level was comparable in both cell lines. As shown in Fig. 6(A), 10 nM DHT reduced the AR content of EFM-19 cells to $67 \pm 4\%$ after 7 days (mean $\pm$ SD of 3 independent experiments).

The intact function of the estrogen receptor in EFM-19 cells was demonstrated indirectly by the induction of the progesterone receptor by 10 nM 17β-estradiol (E_2) [Fig. 6(B)]. In parallel cultures the influence of E_2 on the AR was investigated [Fig. 6(C)]. The level of the AR decreased in estrogen treated cells to $76 \pm 3\%$ of

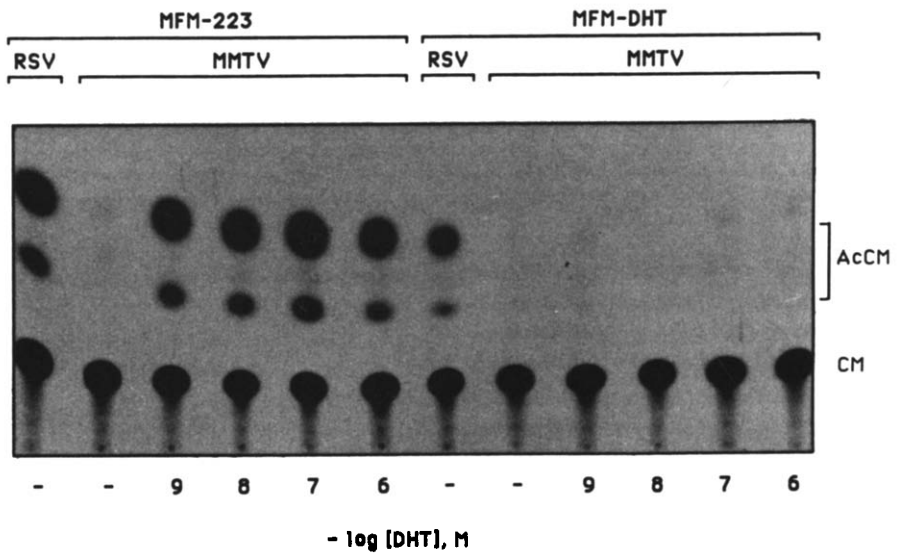

Fig. 5. Influence of increasing concentrations of DHT on the expression of CAT from the MMTV promotor in MFM-223 cells and in MFM-223 cells grown for 17 weeks in the presence of 10 nM DHT (MFM–DHT). The RSV cat construction was also transfected to control the transfection efficiency of the two cell lines. Numbers beneath the autoradiogram indicate the concentration of DHT. Where no hormone was added, a dash (–) appears. The mobility of the substrate chloramphenicol (CM) and the acetylated forms (AcCM) are indicated on the right.

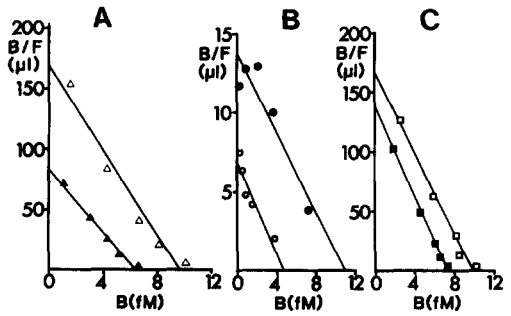

Fig. 6. (A) Down-regulation of AR in EFM-19 cells by 10 nM DHT for 7 days (control: 58,000 sites per cell, $K_d = 0.06$ nM, open triangles; DHT-treatment: 38,500 sites per cell, $K_d = 0.08$ nM, closed triangles). (B) Induction of the progesterone receptor by incubation with 10 nM E_2 for 3 days (control: 31,000 sites per cell, $K_d = 0.71$ nM, open circles; E_2-treatment: 70,500 sites per cell, $K_d = 0.79$ nM, solid circles). (C) Down-regulation of AR by 10 nM E_2 for 3 days (control: 59,000 sites per cell, $K_d = 0.06$ nM, open squares; E_2-treatment: 43,700 sites per cell, $K_d = 0.05$ nM, solid squares). All values are expressed per 100,000 cells, to correct for the growth stimulatory effect of E_2.

the control, as calculated from 3 independent experiments.

Finally, the synthetic progestin R5020 was used to investigate the regulation of the AR level in EFM-19 cells. Experimental cultures were grown for 5 days with 10 nM E_2 to induce high progesterone receptor levels [compare Fig. 6(B)]. The addition of R5020 in the range of 1 nM up to 1 μM yielded a dose-dependent reduction of the AR level (Fig. 7). At the high concentraton of 1 μM a level of approx. 60% of the control was determined. All values were corrected for the actual cell number, as the cell counts were reduced by 10% in the presence

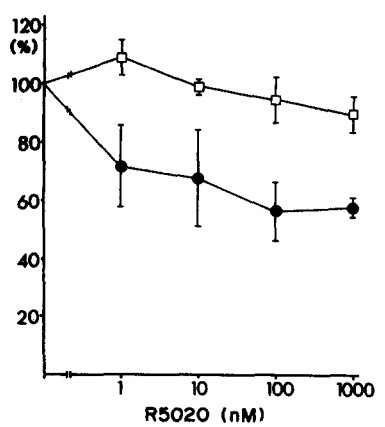

Fig. 7. Progestagenic reduction of AR protein and slight inhibition of cell proliferation. EFM-19 cells were incubated with R5020 in the concentration range 1 nM to 1 μM for 5 days in the presence of 10 nM E_2. The mean cell counts (squares) and numbers of binding sites per cell (circles) were calculated from 3 independent experiments and expressed as percentages of the control ± SD.

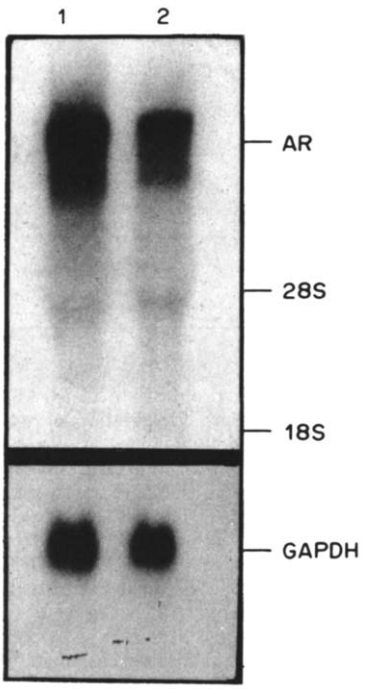

Fig. 8. Northern blot hybridization of AR Poly(A$^+$)RNA from EFM-19 cells (lane 1). Progestagenic down-regulation of AR mRNA (lane 2) was demonstrated after incubating the cells with 1 μM R5020 for 5 days. The experiment was performed in the presence of 10 nM E_2. The blots were rehybridized with GAPDH cDNA to verify the integrity and amount of total RNA loaded.

of 1 μM R5020 (Fig. 7). In EFM-19 cells grown in the absence of estrogen, down-regulation of AR by R5020 was <15% (data not shown).

The down-regulation of the AR by R5020 can also be shown by analyzing the corresponding mRNA in EFM-19 cells. These determinations were based on Poly(A)$^+$RNA, as total RNA from EFM-19 cells yielded only a weak signal in the Northern blot. Down regulation of AR mRNA was found after incubating EFM-19 cells with 1 μM R5020 for 5 days (Fig. 8). The androgen receptor mRNA content was 78% after correction for the GAPDH mRNA content.

Immunocytochemical measurement of the AR

The AR protein is also detectable by immunocytochemical methods using the monoclonal antibody F39.4.1. An intensive nuclear staining was found in both cell lines (Fig. 9). After incubation with androgen, the staining was reduced only slightly. The small variation of the staining intensity in DHT treated cells does not allow a quantitative analysis of the autoregulation of the AR.

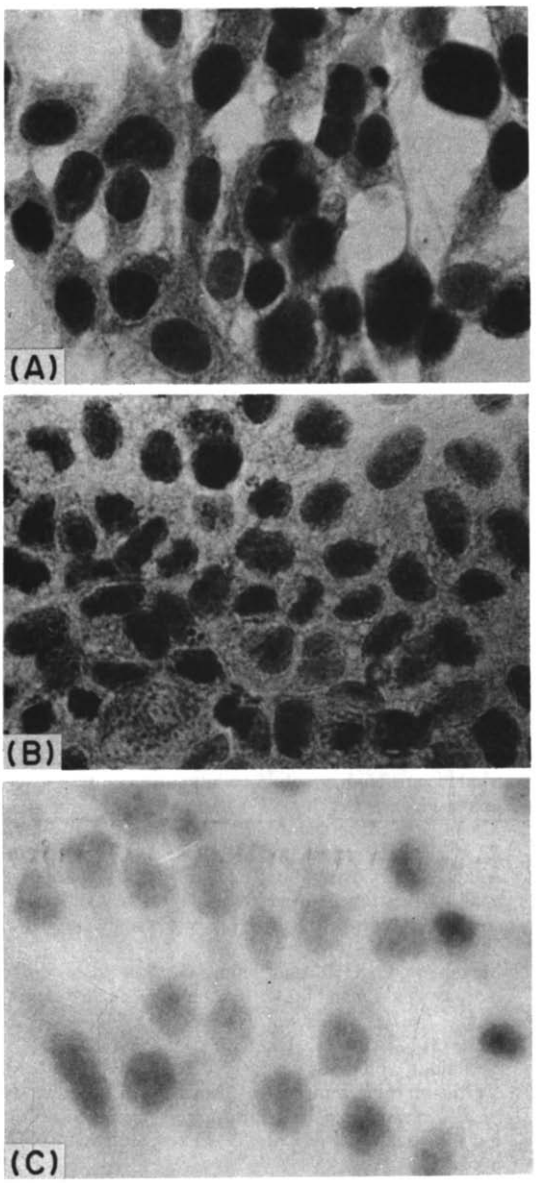

Fig. 9. Immunocytochemical stain of the AR with the monoclonal antibody F39.4.1 in MFM-223 (A) and EFM-19 (B) cells (×160). Negative control of MFM-223 cells (C) without primary antibody.

DISCUSSION

We have determined some of the hormonal factors that influence the regulation of the AR in human mammary cancer cells. Autoregulation seems to be a common phenomenon in the steroid hormone receptor family. Increase of rat prostate AR mRNA after castration, was reversed by injection of testosterone proprionate demonstrating autoregulation of the AR [31]. The glucocorticoid [32], progesterone [33] and estrogen receptors [34, 35] are also regulated by their own ligands. Regulation of the AR by steroid hormones was now investigated in the human breast cancer cell lines MFM-223, characterized by a very high AR level, and EFM-19, which also contains significant levels of estrogen and progesterone receptors. Unlike rat prostate, which is growth-stimulated by androgen, the investigated human mammary carcinoma cells are distinctly inhibited (MFM-223) or unaffected (EFM-19) by DHT in physiological concentrations of up to 10 nM.

Autoregulation of the AR mRNA was comparable in rat prostate and both of the above cell lines. The significant down-regulation of AR mRNA within 6 h in MFM-223 cells suggests a direct regulatory effect on its own gene. In the rat AR gene promotor, TGTYCT elements were found at four positions between −174 and −505 in the promotor region [36]. A glucocorticoid responsive element consists of a palindromic pair of the TGTYCT sequence, separated by three nucleotides [37], and it is known, that this element also mediates progesterone and androgen induction [38]. However, the nonpalindromic sequence might also have some function in steroid hormone responsive promotors [36]. In this respect, it is of interest that TGTYCT sequences are present in the promotors of the androgen dependent prostatic binding protein genes C1 and C2 [39].

AR protein levels were determined by a whole cell binding assay with [^{3}H]R1881. After incubation with 1 nM R1881 for 1 and 6 h the AR remained in a completely exchangeable form [40]. This is a particular feature of the AR. In contrast, estrogen receptors were rapidly depleted of occupied receptor sites, reaching 70% in exchange assays using MCF-7 cells [41]. After the extensive washing procedure used in this investigation, incubation with up to 10 nM DHT for 2 h did not affect the number and affinity of AR sites in MFM-223 cells. Binding to the AR was down-regulated to 57% of the control after incubating the cells with 10 nM DHT for 24 h paralleling the reduction of the equivalent mRNA. Withdrawal of the androgen on day 7 of the incubation was followed by a complete restoration of the AR level supporting the reversible character of this effect. In the LNCaP human prostate carcinoma cell line an increase of AR binding sites and a decrease of AR mRNA after treatment with androgen was observed. The upregulation of AR protein in this cell line can occur principally because of increased translational efficacy and/or stabilization of the protein [42]. The posttranscriptional mechanisms are obviously different in human

mammary and prostate cancer cells, although details of the mechanisms involved remain obscure.

After long-term incubation of MFM-223 cells with DHT, androgen responsiveness and AR content were altered significantly. The inhibitory effect of DHT on the proliferation of MFM-223 cells was completely lost, and in the transfection studies only minimal functional activity of the residual AR was detectable. Yet the AR remained easily demonstrable by immunocytochemistry and binding assays. The ARs, found after long-term treatment with androgen, seem to be functionally inactive, although the binding of androgen is not affected. In human breast cancer tissue, a variant estrogen receptor was detected, which lacked the hormone binding domain and constitutively activated transcription of an estrogen dependent gene [43]. Eventually the binding of the AR to DNA is impaired by a comparable mechanism in MFM–DHT cells.

This study demonstrated the dose dependent reduction of AR by the progestin R5020 in EFM-19 cells, which is maximal after incubation with R5020 at the high concentration of 1 μM. Effective down-regulation requires high progesterone receptor levels found after stimulation with E_2, as demonstrated with EFM-19 cell cultures in the absence of E_2. Androgen binding in MCF-7 and EFM-19 cells was also down-regulated by medroxyprogesterone acetate, which is a progestagenic agent, also binding to the AR and glucocorticoid receptor [20]. R5020 also reduced estrogen receptor mRNA in T47D cells [34], demonstrating uniform regulation of estrogen receptors and ARs by progestins in these mammary cancer cells. Even though R5020 binds to the glucocorticoid receptor with low affinity [11], the involvement of this receptor seems unlikely. Cortisol stimulates the proliferation of EFM-19 cells [44], and the induction of the progesterone receptor by E_2 is necessary for the effective down-regulation of the AR by R5020.

In MCF-7 cells AR was reduced to $< 50\%$ by 2.6 nM E_2 within 2 days [19]. The estrogenic effect on the AR was less pronounced in our experiments with EFM-19 cells. This may be due to the relatively low estrogen receptor level of 7 fmol/mg protein in the EFM-19 cell substrain used. The integrity of the receptor was proven by the inducibility of the progesterone receptor, using the same incubation scheme with E_2.

The AR is strongly regulated by its own ligand. Down-regulation by progestins and to a less extent by estrogens were also demonstrated. These results may contribute to understand the complex interactions of steroid hormones in human breast cancer.

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