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Androgen Receptor Mediated Growth Control of Breast Cancer and Endometrial Cancer Modulated by Antiandrogen- and Androgen-like Steroids

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Androgens are involved in many regulatory processes in mammary and endometrial epithelium, but their role in the development and progression of breast and endometrial carcinoma is poorly understood. Androgen receptors (AR) are found in normal epithelium as well as in more than 50% of specimen from both tumor types. The occurrence of AR is correlated with estrogen and progesterone receptors. Androgen receptor positive cell lines were established during the last few years in our laboratory from malignant mammary (MFM-223) and endometrial (MFE-296) tumors supplementing the small number of androgen-responsive cell lines published so far. In this paper some aspects of the role of androgens in these two types of hormone responsive female cancer are presented. The proliferation of ZR-75-1, MFM-223 and MFE-296 cells is inhibited by androgens. The progestin medroxyprogesterone acetate inhibits the proliferation of estrogen- and progesterone receptor negative MFM-223 cells via the androgen receptor. Some steroid metabolites with distinct estrogenic properties like androst-5-ene-3β,17β-diol possess androgenic properties in this model system. Androgens stimulate the *in vitro* secretion of gross cystic disease fluid proteins by human mammary cancer cells. These proteins are normally found in benign breast cysts *in vivo*. The occurrence of gross cystic disease is correlated with an increased risk of breast cancer. The AR is autoregulated in MFM-223 mammary cancer cells on the protein and mRNA level. In MFE-296 cells with endometrial origin AR protein was increased after incubation with androgens.

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ANDROGEN RECEPTORS IN BREAST AND ENDOMETRIAL CANCER TISSUE

Frequency and biological significance of the receptors for estrogens, progestins, glucocorticoids, and androgens have been investigated intensively during the last decades. AR levels above 20 fmol/mg protein were found in nearly 50% of all breast cancer specimen investigated [1–7]. In a more recent study ARs above 10 fmol/mg protein were found in 85% of 852 tumors investigated [8]. The AR levels are closely correlated with the content of estrogen and progesterone receptors [2, 8–10]. Only in 5–10% of all patients ARs were found in the absence of estrogen and progesterone receptors [8, 11].

The presence of estrogen and progesterone receptors predicts a good response rate of breast cancer to hormonal treatment. As the levels of all steroid hormone receptors are correlated with each other, it is difficult to investigate the influence of the AR independently from the other steroid hormone receptors. There was no significant correlation between the AR content and the response rate to the endocrine therapy of breast cancer patients [12, 13].

Some publications claim that the detection of androgen receptors is correlated with a better prognosis of breast cancer [7, 14]. We could not confirm these data in a group of our own patients. 187 breast cancer specimens were analyzed and 66 (35%) were found to be AR-positive. 155 of these patients were followed clinically (mean observation time: 97.3 months) and we did not observe any differences between the intervals

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from operation to recurrence of the disease or to death of the patients [15].

In endometrial carcinoma tissue ARs were detected in 65–75% of primary tumors [16]. ARs are also found in normal human endometrium at concentrations of 174 fmol/mg protein in the proliferative phase and 156 fmol/mg protein in the secretory phase [16].

ANDROGENS IN THE TREATMENT OF BREAST CANCER AND ENDOMETRIOSIS

Until the 1970s androgens were used in the palliative treatment of breast cancer. Using testosterone propionate and other derivatives remission rates of nearly 30% were achieved [17, 18].

Endometriosis is a benign disease characterized by the spreading of endometrial cells beyond the limits of the uterine cavity. It had been treated successfully with androgens [19]. Yet there is little additional knowledge on the functional role of ARs in human endometrium.

Because of their severe side effects androgens were later replaced by other compounds in the treatment of both diseases. But these results demonstrate that androgens can inhibit the proliferation of mammary and endometrial cells.

ANDROGEN RECEPTOR POSITIVE CELL LINES FROM HUMAN BREAST AND ENDOMETRIAL CARCINOMAS

MFM-223 cells were derived from the pleural exudate of a breast cancer patient [20]. This cell line contains high levels of ARs (160 fmol/mg protein), whereas only low levels of estrogen- (8 fmol/mg protein) and progesterone-receptors (18 fmol/mg protein) were found. The proliferation of MFM-223 cells is strongly inhibited by androgens and is nearly unresponsive to estrogens, progestins and glucocorticoids. The predominance of the AR and the growth control by androgens is the unique feature of MFM-223 cells among the mammary cancer cell lines published so far (Table 1). An androgen resistant subline of MFM-223 cells was selected during continuous incubation with androgen [21]. This cell line was named MFM-DHT (Table 1).

MFE-296 cells represent an AR-positive endometrial cancer cell line [26]. The steroid hormone receptor concentrations were 30, 7 and less than 5 fmol/mg protein of androgen-, progesterone- and estrogen-receptors, respectively. As in the mammary cancer cell line, the proliferation of MFE-296 cells is inhibited by the androgen dihydrotestosterone. The MFE-296 cell line is the only androgen receptor endometrial cancer cell line published so far.

Both cell lines are well suited *in vitro* models to investigate the role of androgens in the control of breast and endometrial cancer growth.

STIMULATORY AND INHIBITORY EFFECTS OF DHT ON THE PROLIFERATION OF HUMAN MAMMARY AND ENDOMETRIAL CANCER CELL LINES

Proliferation of the estrogen responsive human mammary cancer cell lines MCF-7 and EFM-19 is stimulated by high concentrations of the androgen DHT with a maximum at 1 μM [27, 28]. Proliferation of T47D cells is not affected by DHT [29]. Growth inhibition by androgens was found in ZR-75-1 cells *in vitro*. The maximum effect was observed at 1–10 nM DHT and was much lower at 1 μM, showing a biphasic inhibition pattern. As ZR-75-1 cells are stimulated by estrogens, this is probably due to an interaction with the estrogen receptor at this high concentration [30, 31]. Proliferation of MFM-223 cells, which are nearly unresponsive to estrogens, is strongly inhibited by androgens. Significant inhibition was observed already at 0.01 nM DHT. A maximal effect of DHT was found at 1 nM. Higher concentrations did not yield any additional effect [20]. The androgen receptor positive endometrial cancer cell line MFE-296 is also inhibited by androgens [26]. In both cell lines the effect of DHT is antagonized by antiandrogens, this demonstrating the involvement of the androgen receptor. The biological activity of the androgen receptor in MFM-223 and MFE-296 cells was additionally shown by transfection experiments with an androgen responsive mouse mammary tumor virus (MMTV) construct [21, 26].

Table 1. Steroid hormone receptor content and steroid hormone sensitivity of human mammary cancer cell lines

Cell line	AR	ER	PR	Response to Estrogens	Response to Androgens	Ref.
MCF-7	40	100	>300	↑	—	[22]
T47D	3	17	254	↑	—	[23]
ZR-75-1	34	29	43	↑	↓	[24]
EFM-19	50	7	143	↑	—	[20]
MDA-MB-453	250	n.d.	n.d.	—	—	[25]
MFM-223	160	8	18	—	↓	[20]
MFM-DHT	80	n.d.	n.d.	—	—	[21]

("n.d." = not detectable; "—" = no effect).

DUAL ESTROGENIC AND ANDROGENIC PROPERTIES OF ANDROST-5-ENE-3β,17β-DIOL

Androst-5-ene-3β,17β-diol (ADIOL) and 5_{alpha}-androstane-3β,17β-diol (5_{alpha}A) are metabolites of dehydroepiandrosterone and dihydrotestosterone, respectively. Both compounds are known to have estrogenic activity in different experimental systems [32–35]. The stimulatory, estrogen-like effect of these androgen derived steroids is correlated with their binding to the estrogen receptor. Effective concentrations of ADIOL are within the range of physiological female serum levels (1–4 nM) [36]. On the other hand, the mammary cancer cell line MFM-223 is strongly inhibited by ADIOL and 5_{alpha}A in the same concentration range. Receptor studies and competition experiments demonstrated that the inhibitory effects of ADIOL and 5_{alpha}A in MFM-223 cells are mediated by the androgen receptor. ADIOL and 5_{alpha}A possess thus estrogen- and androgen-like properties and can stimulate or inhibit proliferation of human mammary cancer cells. The reaction pattern of mammary cancer cells depends on the steroid hormone receptor content and the growth characteristics of the individual cell line [37].

INHIBITORY EFFECTS OF MPA ON THE PROLIFERATION OF MFM-223 CELLS

The progestin MPA inhibits the *in vitro*-proliferation of several human mammary cancer cell lines. In T47D [38], MCF-7 and EFM-19 cells [39, 40] the inhibitory effect of MPA is mediated by the progesterone receptor. The progestin MPA binds to progesterone-, androgen- and glucocorticoid receptors with high affinity. Androgen- and glucocorticoid receptors may also be involved in the action of MPA. The ZR-75-1 mammary cancer cell line contains receptors for progesterone, androgens and glucocorticoids. All three receptors contribute to the inhibitory effect of MPA in this cell line [41]. As progestins and glucocorticoids do not affect the proliferation rate of MFM-223 cells, this cell line is suitable to investigate androgen-like effects of MPA without involvement of the progesterone- and glucocorticoid receptors. The proliferation rate of MFM-223 cells is strongly reduced by MPA. The dose response curves of DHT and MPA correspond well with the binding affinities of both steroids to the androgen receptor. The inhibition by MPA can be antagonized with antiandrogens. The androgen-resistant subline MFM-DHT was also resistant to MPA. These lines of evidence demonstrate that the inhibition of MFM-223 cell proliferation by MPA is mediated exclusively by the AR [42].

High-dose therapy with the progestin medroxyprogesterone acetate (MPA) is widely used in the palliative treatment of breast cancer [43–44]. During high-dose treatment with 500–1000 mg per day serum levels of more than 250 nM are found. MPA is preferably used in patients with estrogen- and progesterone receptor positive breast tumors, but nevertheless approx 10% of the patients with estrogen- and progesterone receptor negative breast cancer respond to the treatment with MPA. The effect of MPA in these cases was not understood up to now.

The AR-positive cell line MFM-223 demonstrates that MPA can inhibit the cell growth of mammary tumors via the AR in the absence of estrogen- and progesterone receptors. Considering the high serum levels achieved during MPA-treatment, it seems likely that the AR is involved in the action of MPA in some patients suffering from estrogen- and progesterone receptor negative tumors.

REGULATION OF THE ANDROGEN RECEPTOR LEVEL BY STEROID HORMONES

Androgen receptor protein and mRNA are down-regulated by androgens in MFM-223 cells. After incubating MFM-223 cells with 10 nM DHT for 24 h, the androgen receptor content was reduced by 57%. Unlabeled DHT had to be removed from the culture medium prior to the receptor assay. The cell cultures were rinsed four times before [^{3}H]R1881 was added and the remaining traces of unlabeled DHT did not disturb receptor assays and Scatchard analyses. Down-regulation was reversible after withdrawal of DHT. Some MFM-223 cells were propagated with 10 nM DHT for up to six weeks. The androgen receptor content was not restored completely after withdrawal of DHT. It corresponded to 50–60% of the wild-type cells. The antiandrogens cyproterone acetate and hydroxyflutamide did not affect the androgen receptor content of MFM-223 cells [21]. In MFE-296 endometrial cancer cells AR protein concentration increased during incubation with DHT [26].

Androgen receptor mRNA is also autoregulated in MFM-223. Reduction of the specific mRNA was found 6 h after application of 10 nM DHT. After 2, 4 and 25 days androgen receptor mRNA was reduced to 86, 33 and 7%, respectively [21]. Autoregulation was also found in EFM-19, MDA-MB-453 and other mammary cancer cell lines [21, 25].

Regulation of the androgen receptor content by progestins and estrogens was investigated in EFM-19 cells, as this cell line contains estrogen, progesterone and androgen receptors. The effect of the progestin R5020 was tested in the presence of 17β-estradiol to induce high levels of progesterone receptors. 1 μM R5020 reduced androgen receptor mRNA by 22% and the protein level by 40%. After incubating EFM-19 cells for 3 days with 10 nM 17β-estradiol 43,700 binding sites/cell were found (control: 59,000 sites/cell) [21]. Down-regulation of AR by 17β-estradiol was also observed in MCF-7 cells [45].

In the promoter of the androgen receptor gene of the rat TGTYCT-elements were found at four positions

between −174 and −505 [46], corresponding to a half palindrom of the glucocorticoid and androgen responsive element [47]. Autoregulation seems to be a general phenomenon in the family of steroid hormone receptors. The progesterone [48] and the estrogen receptor [49, 50] are also subject to autoregulation.

ANDROGEN INDUCED SECRETORY PROTEINS

In gross cyst fluid of the breast several specific proteins are found. These proteins, named GCDFP-15, -24 and -44 (gross cystic disease fluid proteins), are found at higher concentrations in the fluid of cysts than in blood plasma. The secretion of these proteins is often found in combination with apocrine metaplasia and proliferative changes of breast epithelium [51].

In vitro androgens stimulate the secretion of GCDFP-15, -24 and -44 in T47D cells and of GCDFP-24 in ZR-75-1 cells. Estrogens inhibit the secretion of GCDFP-24 in ZR-75-1 and MCF-7 cells [52–54].

In ZR-75-1 cells estrogens stimulate proliferation and inhibit GCDFP-24 secretion, whereas androgens inhibit proliferation and stimulate GCDFP-24 secretion [53]. In T47D cells GCDFP-15 secretion was also stimulated by androgens but did not respond to estrogens. Androgen dependent secretion seems to be the unique feature of these proteins.

In MFM-223 androgen dependent secretion of GCDFP-15 was equally found. In the endometrial cancer cell line GCDFP-15 expression was not found by polymerase chain reaction (unpublished results).

It has been supposed that a hormonal imbalance with increased androgen levels may be involved in the etiology of the gross cystic disease [55]. In a number of studies the occurrence of gross cystic disease was correlated with an increased risk of breast cancer [51]. This hypothesis is supported by the fact that at least one of these proteins is found in 80% of all breast cancer specimen [56].

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