ONCOLOGY

Androgen Receptors in the Cytosolic Fraction of Malignant Prostatic Tumors and Their Clinical Significance

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The occurrence and mean content of androgen receptors in the cytosolic fraction of prostatic adenocarcinoma depend on the age of patients, stage of tumor anaplasia, tumor differentiation, type of therapy, and the concentration of androgen receptors.

Key Words: *prostatic cancer, androgen receptors*

The therapy of prostatic cancer (PC) is an urgent problem of oncourology. In Russia, the incidence of PC is rather high and tends to increase [2,6]. This is associated with better diagnostic techniques and aging of the male population. Prostatic cancer is the most common cause of death in men over 70 years both in this country and abroad [2,8].

In the early 1940s, C. Huggins and C. Hodgest formulated the concept of a hormone-dependent growth of prostatic tumors and the principles of endocrine therapy. Estrogen therapy of PC developed in the 1940s still remains a valuable clinical tool. However, the choice of patients for this therapy based on histological examination of the tumor, stage of disease, and age of patients is not always adequate [5].

Even at early stages of disease and prognostically favorable histological variants of tumor the occurrence of primary resistance to estrogens is 20-30% [5,6,10]; in some estrogen-treated patients early progression of tumor growth has been observed [2,14]. Therefore, the choice of adequate therapy still remains an important problem for urologists and oncologists.

The discovery of protein receptors for steroid hormones in normal and tumor cells shed more light on the mechanisms of the steroid hormone effects [3,9,16] and provided objective criteria of the choice of PC patients for endocrine therapy, evaluation of their sensitivity to this treatment, and prediction of a possible outcome of the disease [1,13,14,16].

Previously, it was suggested that the absence of cytoplasmic receptors for steroid hormones in PC is one of the causes of tumor resistance to hormonal therapy [10,15]. However, there is controversy over the relationship between the presence and the concentration of androgen receptors (AR) in prostatic neoplasms and their response to hormonal therapy [10,12,17]. This study is an attempt to resolve this controversy.

Our objective was to evaluate the significance of cytosolic AR for prolonged remission, taking into consideration clinical and morphological characteristics of prostatic tumor and type of therapy.

MATERIALS AND METHODS

The study included 86 PC patients aged 47-87 years (mean age 67.2±1.0 years). Diagnosis was confirmed by morphological examination of biopsy material according to the WHO Histological Classification of Prostatic Tumors (Geneva, 1983). Adenocarcinoma was diagnosed in all patients: small acinous (67% of patients), solid-trabecular (30%), and cribriform (3%).

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TABLE 1. Occurrence and Content of Androgen Receptor (AR) in Prostatic Tumors at Different Stage of Anaplasia in Patients of Different Age

Stage of anaplasia	Parameter	Age of patients	
		60 years and younger	70 years and older
$T_{1-4}N_0M_0$	Number of patients	23	22
	AR-positive, %	69.9	77.2
	Mean AR content, fmol/mg protein	59.1±12.4	56.3±13.1
$T_{2-4}N_{1-4}M_1$	Number of patients	27	14
	AR-positive, %	48.1	57.1
	Mean AR content, fmol/mg protein	100.8±18.4*	140.6±23.5*

Note. Here and in Table 2: *$p<0.05$ in comparison with preceding stage.

Transperitoneal needle biopsy was performed by the Franklin—Silverman method under local (0.5% Novocaine) or intravenous anesthesia. The weigh of bioptates varied from 35 to 75 mg.

Prior to determination of the AR concentration the patients received no anticancer therapy. The content of AR in the bioptates was measured as described [11]. The method is based on precipitation of the hormone-receptor complex from the cytosolic fraction with protamine sulfate and a parallel histological examination. 5α-Dihydro[2,3,4,5,6,7-^{3}H]-testosterone (Amersham) was used as a ligand. Nonspecific binding was determined at a 100-fold excess of 5α-dihydrotestosterone (Koch-Light Laboratories). The ligand binding to the progestin receptors was blocked with a 500-fold excess of triamcinolone acetonide (Sigma). Specific binding was expressed in fmol receptor-bound ligand per mg cytosolic protein [3].

The stage of disease was determined by ultrasound examinations of pelvic and abdominal cavities, compute tomography of the prostate, roentgenography of the lungs, pelvic bones, and spine, ascending bilateral lymphangiography, and by radioisotope investigation of the liver and skeletal and lymphatic systems. According to the TNM classification, the patients were categorized as follows: 26 patients with $T_{1-2}N_0M_0$ stage tumors, 20 patients with $T_{3-4}N_0M_0$ tumors, and 40 patients with $T_{2-4}N_{1-4}M_1$ tumors.

According to applied therapy, the patients were assigned into 3 groups: groups 1 included 50 patients (mean age 68.9±2.8 years) receiving only estrogens (consecutive loading doses of honvan and phosphoestrol, 6-8 g for 5-6 month and supportive therapy with conventional doses of chlortrianisene, microfollin, estradurin, and synoestrol); group 2 patients (n=27, mean age 64.4±2.1 years) received radiation therapy (total dose 62-72 Gy on primary tumor and 45 Gy on regional lymph node) in combination with estrogens (synoestrol, microfollin, phosphoestrol, and chlortrianisene); and group 3 patients (n=9, mean age 59.8±2.6 years) received 6 cycles of estrogen and chemotherapy according to the following scheme: 500 mg/m^2 5-fluorouracil (days 1 and 8) and 50 mg/m^2 adriamycin and 600 mg/m^2 cyclophosphane on day 1 intravenously. Synoestrol was administered intramuscularly in a dose of 80 mg during the first 2 months and 60 mg during two other months in combination with chemical agents, after which the patients continued to take chlortrianisene in a daily dose of 24-36 mg. The treatment was discontinued in 2 patients due to progression of PC.

Remission was determined as the period from the beginning of therapy till the appearance of objective or subjective signs of PC progression: enlargement and/or appearance of nodes in the prostate, new metastases and/or progressive meta-

TABLE 2. Occurrence and Mean Levels of Androgen Receptors (AR) in Prostatic Tumors Depending on the Degree of Tumor Differentiation and Stage of Anaplasia

Stage of anaplasia	Parameter	Degree of tumor differentiation	
		highly differentiated	moderately and poorly differentiated
$T_{1-4}N_0M_0$	Number of observations	33	13
	AR-positive, %	81.8	23.1
	Mean AR content, fmol/mg protein	44.9±7.1	168.0±10.5
$T_{2-4}N_{1-4}M_1$	Number of observations	15	25
	AR-positive, %	53.3	48.0
	Mean AR content, fmol/mg protein	132.9±23.7*	121.5±19.8*

TABLE 4. Duration of Remission in Patients with Prostatic Cancer Treated with Radiotherapy and Estrogens Depending on the Presence and Content of Androgen Receptors (AR) in Tumor, Degree of Tumor Differentiation and Stage of Anaplasia

Degree of tumor differentiation	Stage of anaplasia	Duration of remission, month			
		presence of AR		content of AR, fmol/mg protein	
		AR-negative	AR-positive	10-100	>100
Highly differentiated	$T_{1-4}N_0M_0$	29.3±8.6	41.5±9.3	41.5±9.3	-
	$T_{2-4}N_{1-4}M_1$	19.0±8.5	24.0±5.7	32.0 и 27.0	16.0
Moderately and poorly differentiated	$T_{1-4}N_0M_0$	27.6±3.7	14.6±3.7	20.3±6.3	17.0
	$T_{2-4}N_{1-4}M_1$	36.0	19.0±7.2	23.5±8.2	14.5±8.3

stasizing into the lymph nodes or bones, impaired urination, and pain.

Prognostic validity of the cytosolic AR concentration was determined retrospectively relative to the duration of remission. The patients received different primary therapies irrespective on the presence or absence of AR in the tumor.

RESULTS

Androgen receptors were identified in 51 out of 86 biopsies (59.6%). The receptor content varied from 10 to 238 fmol/mg protein. In 22 biopsies (43.1%) it varied from 10 to 49 fmol/mg protein, in 12 biopsies (23.5%) from 50 to 100 fmol/mg, and in 17 biopsies (33.4%) it was higher than 100 fmol/mg.

As Table 1 shows, the occurrence of AR was the highest in prostatic tumors of patients over 70 years, being much lower at different stages of PC. There was no relationship between the AR concentration and age of patients. However, a significant relationship between the AR concentration and the stage of disease was revealed in patients below 69 and above 70 years. The concentration of cytosolic AR was lower at the early stages of PC (Table 1).

Table 2 shows the relationship between the occurrence of AR, tumor differentiation, and stage of disease. Highly differentiated tumors were identified in 48 patients (55.5%). Thirty-eight patients (44.2%) developed moderately and poorly differentiated tumors. The occurrence of AR in highly differentiated tumor was considerably higher that in moderately and poorly differentiated tumors: 72.9 *vs.* 39.5%. It was the highest (81.8%) in highly differentiated neoplasms at $T_{1-4}N_0M_0$ stage, while in moderately and poorly differentiated tumors at the same stage of anaplasia it was much lower (23.1%). As the disease progressed, the number of highly differentiated tumors containing AR decreased, while that of moderately and poorly differentiated tumors with AR increased.

The mean content of AR in highly differentiated PC at $T_{1-4}N_0M_0$ stage was significantly lower than that at $T_{2-4}N_{1-4}M_1$ stage: 44.9±7.1 *vs.* 132.9±23.7 fmol/mg protein, $p<0.05$. However, the AR content in moderately and poorly differentiated tumors was higher at early stages compared with that at late stages: 168.0±10.5 *vs.* 121.5±19.9 fmol/mg protein.

Our findings indicate that there is a relationship between the occurrence and concentration of AR, on the one hand, and the degree of tumor differentiation and the disease stage, on the other.

Retrospective analysis of the duration of remission relative to the type of therapy and the presence and content of AR was performed in 79 patients with PC. The results are summarized in Tables 3 and 4.

In 13 (26%) out of 50 estrogen-treated patients (group 1), tumors proved to be primarily resistant to estrogens. In 12 patients with estrogen-resistant tumors, the disease was diagnosed at $T_{2-4}N_{1-4}M_1$ stage, 53.7% of the tumors being moderately and poorly differentiated. Androgen receptors were not found in 61.5% of the tumors, while the AR content in all other tumors (38.5%) was >100 fmol/mg protein.

Remission was the longest in estrogen-treated patients with highly differentiated carcinomas at the early stage of anaplasia ($T_{1-4}N_0M_0$) and when the AR content was lower than 100 fmol/mg protein.

Remission was the shortest in patients with highly differentiated tumors containing no AR or with AR content >100 fmol/mg protein, irrespective of the stage of PC (Table 3). A tendency toward a longer remission was observed in estrogen-treated patients with moderately and poorly differentiated adenocarcinomas at $T_{1-4}N_0M_0$ stage and AR content lower than 100 fmol/mg protein (16.4±5.1 months, Table 3).

Thus, in PC patients receiving estrogen therapy remission was long at the early stage of disease ($T_{1-4}N_0M_0$), high degree of tumor differentiation, and AR content lower than 100 fmol/mg protein.

The duration of remission was recorded in 23 out of 25 patients receiving radiotherapy in combination with estrogens (group 2). It lasted 26.8±3.1 months. Two patients with PC at $T_{2-4}N_0M_0$ stage were excluded from the analysis, because this type

TABLE 3. Duration of Remission in Patients with Prostatic Cancer Treated with Estrogens Depending on the Presence and Content of Androgen Receptors (AR) in Tumor, Degree of Tumor Differentiation and Stage of Anaplasia

Degree of tumor differentiation	Stage of anaplasia	Duration of remission, month			
		presence of AR		content of AR, fmol/mg protein	
		AR-negative	AR-positive	10-100	>100
Highly differentiated	$T_{1-4}N_0M_0$	13.1±2.8	22.6±4.7	24.9±5.0	6.0±1.5
	$T_{2-4}N_{1-4}M_1$	3.5±1.1	11.0±4.1	18.3±2.5	6.3±2.3
Moderately and poorly differentiated	$T_{1-4}N_0M_0$	11.0±3.5	12.5±4.1	16.4±5.1	10.2±1.4
	$T_{2-4}N_{1-4}M_1$	5.5±1.6	7.0±2.7	8.4±2.8	4.6±1.9

of therapy stimulated tumor progression. It should be noted that in one of these patients tumor contained no AR, while in the other the AR content was higher than 100 fmol/mg. Both tumors were poorly differentiated. Remission was the longest in patients with highly differentiated tumors at $T_{1-4}N_0M_0$ stage containing cytosolic AR (41.5±9.3 months). In patients with moderately and poorly differentiated tumors, no significant relationship between the duration of remission and the presence of AR receptors was revealed.

Thus, radiotherapy in combination with estrogen and chemotherapy is more effective in patients with highly differentiated, early stage ($T_{1-4}N_0M_0$) tumors containing AR.

Moderately and poorly differentiated tumors at $T_{2-4}N_{1-4}M_1$ stage predominated (66%) in group 3 (estrogen and chemotherapy). Cytosolic AR were identified in 5 tumors, the AR content varying from 20 to 57 fmol/mg protein. This type of therapy stimulated progression of PC in two patients. In one case the tumor contained no AR, while in the other the AR content was 35 fmol/mg protein. The duration of remission in 6 patients of this group varied from 4 to 14 months (8.2±1.5 months). In half of the cases tumors contained cytosolic AR receptors. The longest remission (40 months) was achieved in one patient (AR content 48 fmol/mg protein). However, it is premature to make any conclusion concerning the validity of the AR in patients receiving chemotherapy and estrogens.

From our results it can be concluded that:

- The occurrence and mean contents of cytosolic AR in prostatic tumors are related to the age of patients, stage of disease, and degree of tumor differentiation.
- In contrast to moderately and poorly differentiated tumors, among which AR-negative neoplasms or those with the AR content >100 fmol/mg protein prevail, the occurrence of AR is higher in highly differentiated tumors, the AR content varying from 10 to 100 fmol/mg protein.
- The duration of remission in patients with PC depends on the stage of disease, degree of tumor differentiation, type of therapy, and the presence and content of androgen receptors.
- Remission is longer in patients with highly differentiated tumors at $T_{1-4}N_0M_0$ stage and AR content 10-100 fmol/mg protein than in patients with AR-negative tumors or tumors with AR content >100 fmol/mg protein.
- We found no relationship between the duration of remission in patients receiving radiotherapy in combination with estrogen therapy and the presence and content of AR in prostatic tumors.

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