ONCOLOGY

Steroid Receptor Assay in Various Tissues and Tumors

Z. V. Kuz'mina, V. A. Shatskaya, and Z. C. Smirnova

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Four types of steroid receptors were assayed in 1715 tumors of various localization and in 101 normal tissue samples from the mammary gland, uterus, and endometrium; tissue-specific distribution of steroid receptors was demonstrated. Expression of steroid hormones (especially estrogen receptors) is enhanced in proliferating compared with resting tissues and in hormone-sensitive tumors compared with adjacent normal tissue. It is assumed that hormone sensitivity of the tumor reflects hormone sensitivity of parental organ.

Key Words: *steroid receptors; endometriosis; tumors*

Synthetic hormone preparations are now widely used in clinical practice. However, these preparations, as well as other types of endocrine therapy, are ineffective in some patients. In light of this, precise laboratory tests for hormone sensitivity are necessary.

Steroid receptor assay is the most valid test for hormone sensitivity. In some countries this test became a routine procedure. However, it is sometimes difficult to determine receptor content in metastases during tumor progression. Since affected tissues and organs sometimes cannot be examined, hormone sensitivity of metastases is assessed by the content of steroid receptors in primary tumors of lymph nodes. This is a relative estimation, since the question remains unanswered as whether receptor assortment in metastases of untreated patients is identical to that of primary tumors. Many investigators reported variability of receptor population in metastases, which can be associated with tumor malignization and effects of adjacent tissues.

We assume that disseminated genital endometriosis can be used as an adequate model for the study of steroid receptors in tumors. The mechanism of progression of this benign tumor resembles metastatic process. Moreover, hormone-sensitivity of endometriotic foci has been demonstrated, which is consistent with the presence of estrogen (ER) and progesterone (PR) receptors in these structures [11].

The aim of the present study was to evaluate receptor status of endometriotic foci in disseminated genital endometriosis and to compare these data with steroid receptor content in normal tissues and tumors in these organs.

MATERIALS AND METHODS

We present here clinical and experimental data obtained at N. N. Blokhin Oncology Research Center over 15 years. To answer the question whether morphologically homogeneous heterotopic foci exhibit the same steroid receptor activity, we assayed different tissue samples from 54 patients with disseminated endometriosis. Additionally, 1715 tumors of various localizations were analyzed. We also analyzed normal tissue samples from mammary gland (22 patients), ovaries (32 patients), and myometrium (57 patients). Some experiments were carried out on female CBA mice (2-month-old and older, 18-20 g). The contents of four types of cytoplasmic steroid receptors:

N. N. Blokhin Oncology Research Center, Russian Academy of Medical Sciences, Moscow

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ER, PR, glucocorticoid receptors (GR), and androgen receptors (AR) were measured by competitive binding assay using tritiated ligands [7]. Free and bound hormones were separated using dextran-coated charcoal. For ER, GR, and AG, tissues with ^{3}H-hormone binding exceeding 10 fmol/mg cytosol protein were considered as receptor-positive. For PG, this boundary was 20 fmol/mg protein for uterine tumor and endometriosis and 10 fmol/mg for other tissues.

RESULTS

Progesterone receptors predominated in endometriosis samples of various locations. The incidence of PR positivity varied from 95% (endometriotic lesions of the corpus and isthmus of the uterus, retrocervical space, and ovaries) to 25% (endometriotic lesions in the urinary bladder). These receptors were found in endometriotic implants in the vagina, rectum, ureter, cervix, broad ligament, small intestine, and in 1 out of 3 samples from the vagina and sigmoid colon [5].

The highest concentration of PR was noted in endometriotic tissues in the body of the uterus and adjacent tissues, while remote foci were characterized by lower PR concentrations. Estrogen receptors occurred in no more than 50-56% samples and were equally distributed. Glucocorticoid receptors and AR were much less abundant. Their concentrations were similar in endometriotic implants from various organs only slightly exceeded the boundary of receptor posi-tivity. Expression of these receptors was most pronounced in endometriotic lesions of the ovaries, uterus, and isthmus. Generally, all types of steroid receptors were most frequent in endometriotic samples from the uterus and less abundant in other tissues and organs (Table 1).

Thus, expression of steroid receptors in endometriotic implants depends primarily on their location: the more distant the lesion from the uterus, the lower its receptor activity. It can be assumed that hormone sensitivity of endometriotic lesions correlates with that of parental organ.

This assumption can be extended to malignant tumors. Receptor analysis of normal and tumor samples from various organs revealed a tissue specificity in the distribution of steroid receptors. For instance, PR were expressed in normal and tumor tissues of the uterus, GR in normal ovaries, melanoma, and endometrial cancer, while ER and AR in endometrial and

TABLE 1. Steroid Receptors in Tumors of Various Location and Normal Tissues (fmol/mg protein, *M±m*)

Tumor	Number of patients	ER	PR	GR	AR
Endometrial cancer	149	131.6±12 (75)	326.6±29 (81)	77.1±8.6 (57)	33.5±3.5 (49)
Glandular polyps	20	246.8±31.8 (95)	223.7±42.1 (95)	34.2±6.1 (32)	33.7±5.3 (50)
Endometriosis of the uterus	25	26.0±7.1 (40)	188.9±17.0 (80)	15.9±8.4 (50)	16.7±8.4 (66)
Normal myometrium	57	34.1±7.9 (80)	178.8±30.2 (70)	—	—
Myoma	21	61.9±15.4 (73)	190.5±67.9 (60)	—	—
Breast cancer	853	55.7±5.8 (64)	123.1±12 (61)	52.3±5.9 (56)	36.5±9.8 (46)
Premenopause (breast cancer)	291	43.8±5.2 (59)	117.0±11.6 (64)	52.2±6.8 (59)	39.2±14.5 (50)
Postmenopause (breast cancer)	562	70.4±6.9 (68)	130.0±13.9 (59)	48.5±3.0 (53)	33.6±8.1 (42)
Mammary gland fibroadenoma	70	51.4±9.8 (57)	62.6±8.5 (60)	54.5±9.1 (47)	20.6±3.4 (6)
Fibrocystic breast disease	185	56.9±10.6 (45)	58.6±13.5 (43)	46.6±9.0 (44)	22.5±5.0 (10)
Normal mammary gland	22	19.4±2.8 (42)	17.8±2.5 (33)	22.1±8.4 (40)	—
Ovarian cancer	41	29.5±2.2 (56)	25.9±2.3 (28)	38.9±4.1 (62)	25.8±7.9 (27)
Endometriosis of ovaries	41	13.9±2.5 (42)	59.2±8.4 (83)	43.1±8.0 (69)	19.5±6.0 (25)
Normal ovaries	32	16.4±3.2 (26)	68.1±4.8 (77)	74.6±8.8 (89)	17.2±1.0 (51)
Cancer of the colon and rectum	77	27.5±2.1 (30)	22.1±5.7 (19)	37.8±2.8 (38)	23.4±3.2 (15)
Rutal endometriosis	24	24.6±3.2 (17)	27.8±13.6 (67)	20.3 (14)	14.6 (17)
Kidney cancer	56	36.9±11 (22)	32.5±11 (14)	81.8±10.4 (70)	15.1±2.3 (3)
Esophageal cancer	57	19.3±2.8 (26)	22.7±5.1 (19)	44.0±6.8 (53)	15.5±3.5 (2)
Melanoma	142	39.1±6.0 (25)	23.6±4.3 (18)	63.9±5.0 (57)	14.0±1.1 (4)
Lung cancer	44	11.8±1.9 (80)	36.7±0.3 (8)	26.8±2.9 (63)	—

Note. % of receptor positive tissue is shown in parentheses.

TABLE 2. Concentration of Steroid Receptors Assay in Mouse Cervical Cancer Samples with Different Inoculation Sites (fmol/mg protein, *M±m*)

Day after inoculation	Site of inoculation	ER	PR	GR	AR
11	Intrauterine	46.7±21	76.0±21	30.1±2.3	0
	Subcutaneous	6.1±0.3	13.4±0.3	7.9±5.5	0
14	Intrauterine	31.2±6.7	59.5±0.9	48.2±9.7	4.0
	Subcutaneous	6.0±5.0	11.3±4.7	10.8±6.7	0
18	Intrauterine	8.5±3.1	19.3±14	21.6±18	0
	Subcutaneous	3.6±1.5	7.1±6.0	32.5±2.8	1.4

mammary gland tumors. The maximum receptor activity was found in normal and transformed uterine tissues, glandular uterine polyps, and mammary gland tumors, while normal ovaries and ovarian tumors were characterized by a lower steroid receptor concentration. ER in other organs and tissues were moderate in number and equally distributed. We have previously demonstrated that expression of ER in tumors surpasses that of normal parental tissues [1-4,6,7].

The assumption on the correlation between hormone sensitivity of tumors and normal parental tissues was confirmed in experiments with intrauterine and subcutaneous implantation of mouse cervical tumors. As seen from Table 2, expression of steroid receptors strictly depended on the site of implantation. Receptor activity was higher in intrauterine implants.

Numerous experimental and clinical data demonstrate the absence of a clear-cut correlation between receptor status of the tumor and the efficiency of endocrine therapy. For instance, hormone therapy is effective in 50-60% patients with ER-positive mammary gland tumors (50-60% of tumors) and, surprisingly, in 8-10% patients with ER-negative tumors.

Some investigators reported a correlation between the efficiency of treatment and a certain type of steroid receptor, in particular PR for the uterine and ER or ER+PR for mammary gland tumors; however the role of different steroid receptors in some tissues remains unknown. Moreover, various tissues express antiestrogen-binding proteins, which not always correlate with the presence of ER and whose function also remains unclear [14].

Steroid receptor assay is associated with serious methodological difficulties, in particular, tissue and cell heterogeneity [10]. Free steroid receptors can transit from the nuclear to cytoplasmic fraction upon homogenization. The concentration of steroid receptors depends on physiological state of the organism (pre- and postmenopause, lactation). It has been reported that the rate of detection of receptor-positive tumors varied from one laboratory to another and even in the same laboratory despite the unified assay procedures used [13]. These factors stimulate the development of more reliable methods for evaluation of tumor hormone sensitivity. The most promising approach might be detection of estrogen-induced marker proteins in the plasma or urine in the dynamics of tumor process [9,12].

Formation of hormone-receptor complex is a principal stage in the genetically determined mechanism of action of steroid hormones. This process is negligible in normal resting tissues and highly pronounced in target tissues and tumors. In particular, expression of ER in tumors is considerably enhanced. This is consistent with published data on shorter recurrence-free survival associated with high ER levels in tumors [15].

Thus, steroid receptors are present practically in all tissues in the organism, but their distribution is tissue-specific. Expression of steroid hormones (especially, ER) is enhanced in proliferating tissues. In hormone-sensitive tumors, the concentration of steroid receptors is higher than in normal tissue and, apparently, reflects hormone sensitivity of parental organ.

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