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Soluble and nuclear type I and II androgen-binding sites in benign hyperplasia and cancer of the human prostate

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Summary. This paper presents an approach for the assessment of the androgen receptor (AR) status in benign prostatic hyperplasia (BPH) and prostate cancer (PCa) tissues. Evaluation of AR was carried out in both soluble and nuclear fractions by a standard competition method, using tritiated mibolerone as radioligand. Based on our experience with breast and endometrial cancer, this approach focused on both type I (high affinity, low capacity) and type II (reduced affinity, higher capacity) binding sites, aiming mainly at establishing a putative "functional" receptor mechanism, i.e., the presence of type I AR in both cytosol and nucleus. Ancillary studies were carried out to exclude a potential overestimation of the AR content by interference with other steroid receptors, namely, progesterone (PgR) or glucocorticoid (GcR) receptors. Results showed that the interaction by PgR or GcR upon AR measurement was not relevant. The distribution of AR, namely the percent of positivity either in a single or in both cell compartments, was not significantly different in BPH ($N = 32$) or PCa ($N = 24$) tissues. For type I binding, the percent of positivity in both soluble and nuclear fractions (i.e., the "functional" AR status) was very close to that observed for other endocrine-related tumors, like breast cancer. Concentrations of type I AR appeared significantly higher in PCa than in BPH tissues; this was true for both soluble and nuclear fractions. In contrast, no significant difference was found in type II AR concentrations in either cell fraction. Nuclear type I AR proved to be positive in the great majority (more than 90%) of both PCa and BPH specimens; thus, this study does not support the hypothesis that nuclear AR may have a prognostic value, as previously suggested. Until long-term follow-up data on PCa patients are available, the predictive value of AR status, as estimated by this approach, cannot be assessed; however, in parallel with the studies carried out on estrogen receptor status in breast cancer patients, we suggest that a "functional" AR status is better indicated by the presence of type I binding in both cell fractions.

Key words: Androgen receptors – Type I and II binding sites – Prostate – Hyperplasia – Cancer

As established by Huggins and Hodges in 1941 [1], both benign prostatic hyperplasia (BPH) and prostate cancer (PCa) growth are under the control of steroid hormones. Since the 1970s, research has mainly focused on the androgen receptor (AR) content of prostatic tissues.

Initially, in 1971, Hansson and colleagues [2] provided convincing evidence for the presence of AR in the human prostate. Subsequently, AR has been documented in both normal and diseased prostate gland [3–6].

However, most clinicians are still at a loss to follow advances in research on AR and understand their clinical value. Some investigators have reported that their presence has prognostic value and can predict response to endocrine treatment in PCa patients [7–9]. However, results from AR assays are often not uniform or even conflicting; differences in both techniques and methodological approaches may be regarded as a critical step in accounting for these discrepancies [10, 11].

The intracellular distribution of AR has also been evaluated in recent years. It has been suggested that nuclear AR (ARn) is a better discriminant than cytoplasmic AR (ARc) in predicting the clinical response of PCa patients [12–13], but this has not been universally accepted.

Cytochemical assays of the AR content in prostatic tissue using either fluorescent dyes or monoclonal antibodies seem to indicate that intracellular binding sites for androgens are present in greater amounts than predicted by biochemical assays [14]. It is conceivable that AR sites recognized by immunocytochemistry are different from those identified by the classical dextran-coated charcoal (DCC) method [14]. This may be due to the presence of type II receptors, which show high capacity of hormone binding but reduced affinity, as opposed to type I receptors with higher affinity but lower binding capacity [15]. Little is known about the distribution of type I and II

receptors in prostatic tissues, although they have been clearly demonstrated in breast cancer [16]. However, recent investigations on canine prostate cells have documented the presence of different AR types, which can be distinguished on the basis of their characteristics of binding affinity, capacity, and saturability [17, 18].

As previously suggested by different research groups [19–21], including our own [22, 23], a "functional" receptor status coincides with the presence of type I binding sites in both soluble and nuclear fractions; it is postulated that this corresponds to an unimpaired receptor mechanism, as the precise significance of type II steroid receptors has not been clearly established. This assumption is well supported by follow-up studies of both breast and endometrial cancer patients, where such an approach has provided a valuable prognostic factor [24–27] and allowed an improved prediction of response to endocrine therapies [28–30].

Thus, receptor studies should not only aim at investigating AR concentrations in prostatic tissue, but also at establishing their putative "functional" status. The latter may depend not only on their intracellular localization, i.e., in cytoplasm and nucleus, but also on their behavior as type I and II receptors.

The main goal of the present study was to ascertain the presence of type I and II AR in both soluble and nuclear fractions of human BPH and PCa tissues. Since we have shown that the AR content of both types does not differ significantly in prostatic specimens obtained by either open surgery or transurethral resection (TUR) [31], prostatic tissues were employed for AR assays irrespective of the surgical procedure adopted. Furthermore, the possible binding of mibolerone, the new ligand used for AR assay, to both glucocorticoid and progestin receptors was also taken into account.

Materials and methods

Patients

Overall, 56 samples of prostatic tissues, 24 of PCa and 32 of BPH, were obtained by TUR or open surgical removal. PCa patients were aged 59 to 84 years (mean 74.8), while BPH patients were aged 57 to 82 (mean 68.4) years. The nature of the prostatic disease was assessed by histological examination prior to receptor assay. Tissues with overt signs of prostatitis were excluded from the study.

Specimens were analyzed for AR type, concentration, and intracellular distribution. The relative value of type I and II AR was calculated separately in both soluble and nuclear compartments.

Androgen receptor assays

BPH and PCa specimens were either processed immediately after the surgical intervention or stored at −20°C in a buffered system (Sucrose 250 mM/Hepes 10 mM/glycerol 50% v/v, pH 7.4); previous experience has shown that both methods yield identical results [32]. Before the assay, stored tissues were reconditioned in sucrose buffer (Sucrose 250 mM/Hepes 10 mM/MgCl 1.5 mM, pH 7.4) for 20 min. Fragments (50–200 mg) of fresh or reconditioned tissues were homogenized in buffer (Hepes 10 mM/EDTA 1.5 mM/sodium molybdate 10 mM/dithiothreitol 5.0 μM/glycerol 30% v/v, pH 7.4) using a glass/glass homogenizer. The homogenate was then centrifuged at 800 *g* for 5 min to separate the nuclear (pellet) from the soluble (supernatant) fraction. The nuclear pellet was resuspended three times in buffered saline to remove any possible soluble contaminants.

Aliquots (150 μl) of each cell fraction were incubated overnight at 4°C against increasing concentrations (from 0.1 to 5 nM) of the radioligand, namely, tritiated mibolerone ([17α-methyl-^{3}H]-mibolerone, Amersham). This novel synthetic androgen seems to be more specific and selective than methyltrienolone (R1881) [33], which has been more commonly used for this purpose. Competition studies were carried out using a 100-fold excess of cold mibolerone. A constant concentration (10^{-7} M) of unlabelled triamcinolone acetonide (TA) was also used to hamper mibolerone binding to glucocorticoid receptors.

In a separate series of experiments, excess (10^{-7} M) of TA was used alone or in addition to the same concentration of cold ORG2058, a synthetic progestin highly specific for progesterone receptors [34], in order to measure possible binding of mibolerone to either progestin and/or glucocorticoid receptors.

Soluble fraction. After incubation, 0.9 ml of HE buffer (Hepes 10 mM/EDTA 1.5 mM) was added to the cytosol fraction to stop the reaction by dilution. Each tube was treated by addition of 0.5 ml of DCC suspension, mixed for 15 min to strip all the unbound radioligand and centrifuged at 4°C for 5 min. Aliquots (1 ml) of the deriving supernatant were then placed in scintillation vials and counted for radioactivity in a β-counter.

Nuclear fraction. Nuclear samples were aliquoted (100 μl) and set in tubes containing 5 ml of saline. Removal of all the unbound radioligand was achieved through filtration on premoistened Whatman GF/C glass fiber filters, using a Millipore apparatus. The Millipore funnel was washed once with saline; filters were removed, placed in scintillation vials, and dried overnight at room temperature. Finally, scintillation cocktail was added and the vials counted for radioactivity.

Analysis and expression of data. Following Scatchard analysis for plotting values, a 9 + 3 points competition curve was constructed, yielding both receptor molar concentrations and dissociation constant (Kd) values. Generally, type I receptors are well represented by the first part of the curve, while type II binding is evidenced at ligand concentrations $\geqslant 6.0 \times 10^{-10}$ M, apart from limited overlapping. Kd values within 1.0 nM for defining type I AR were similar to those reported by other authors [35]. For type II AR cutoff values were arbitrarily chosen. The Kd cutoff range was quite distinct from that reported for testosterone-estradiol-binding globulin (TEBG) [36].

Receptor concentrations were expressed as fmol/mg protein and fmol/mg DNA. Protein and DNA tissue content were determined by the classic Lowry [37] and the modified Burton [38] methods, respectively. The use of DNA as a reference point for expressing the results of AR assays appears to be preferable on the basis of both our experience [22] and other reports suggesting that receptor content is independent of the DNA content of tissues [11].

Statistics

Chi-square test, Fisher's exact test, two-tailed Student's *t*-test and Wilcoxon test (95% confidence limits) were used for the statistical comparison of results.

Results

Concerning the possibility that the radioligand mibolerone binds to both progesterone and glucocorticoid receptors, the results shown in Table 1 illustrate the crude

Table 1. Crude cpm values of mibolerone binding in soluble and nuclear fraction of adjacent sections of human prostate tissue[a]

Soluble		Nuclear	
(A)	(B)	(A)	(B)
43.1	36.0	42.1	39.1
62.6	46.5	87.1	64.0
116.3	93.7	140.8	111.6
149.5	163.5	165.1	143.7
317.1	294.8	274.7	230.6
570.3	493.9	469.8	443.7
599.8	574.0	448.7	418.9
802.9	764.0	536.2	560.2
1693.8	1398.6	758.7	845.2

[a] Adjacent sections of the same human benign prostatic hyperplasia tissue were used. Values represent the mean of 14 experiments carried out in the presence of triamcinolone acetonide alone (A) or triamcinolone acetonide plus ORG2058 (B) equimolar excesses (10^{-7} M). Each line represents the reading (crude cpm) obtained using increasing concentrations (from 0.1 to 5.0 nM) of tritiated mibolerone

Table 2. Percent distribution of type I and II androgen receptor (AR) status in human benign prostate hyperplasia (BPH) and prostate cancer (PCa) tissues

	Type I AR			Type II AR	
	S	N	S + N	S	N
BPH ($N = 32$)	53.1 (17)	96.9 (31)	50.0 (16)	59.4 (19)	68.8 (22)
PCa ($N = 24$)	54.2 (13)	91.7 (22)	45.8 (11)	50.0 (12)	75.0 (18)
Statistics (P value)	NSD (0.85)	NSD[a] (0.39)	NSD (0.97)	NSD (0.67)	NSD (0.83)

S, soluble; N, nuclear fraction; S + N indicates the presence of type I AR in both cell fractions, i.e., a putative "functional" receptor status. Statistics: chi-square and [a] Fisher's exact tests. NSD, No significant difference

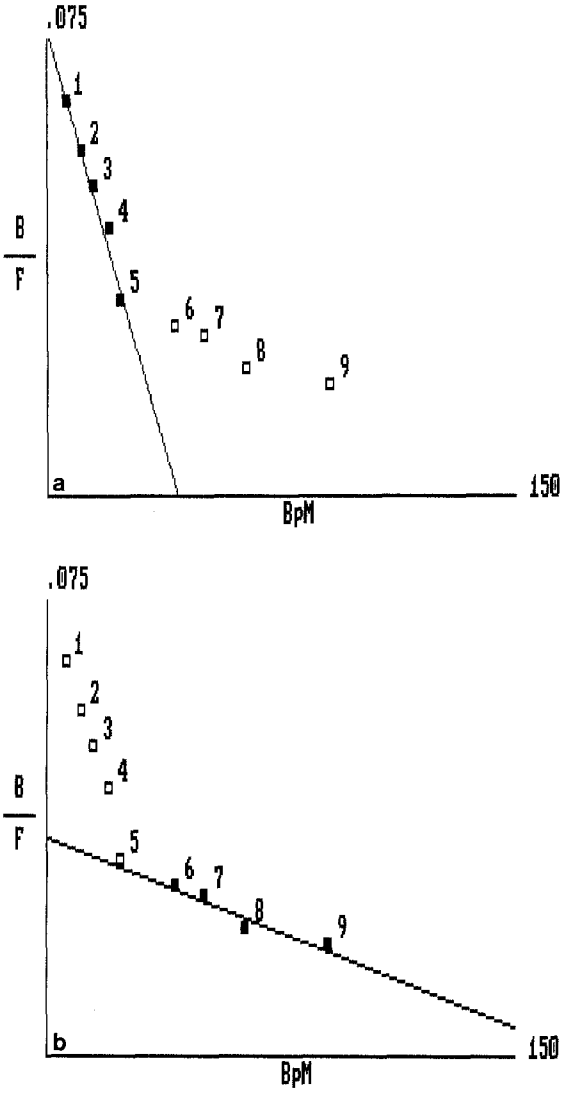


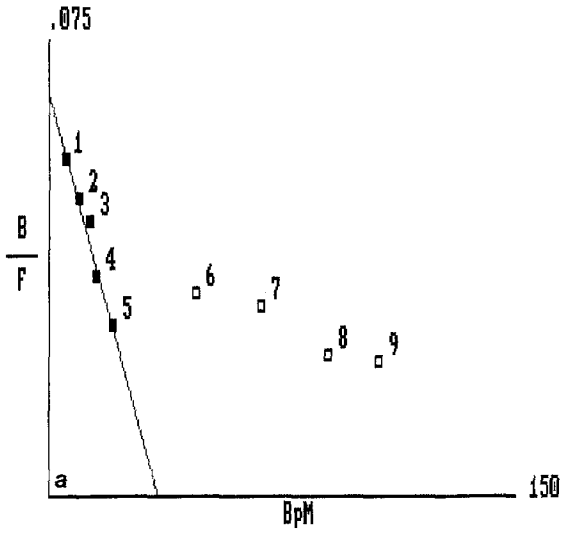
Fig. 1. a, b. Scatchard plots of type I (**a**) and type II (**b**) androgen receptors in soluble fraction of a prostate adenocarcinoma tissue. Nine points at various concentrations of radioligand mibolerone plus three competition points with excess cold mibolerone were used. B/F = bound over free; BpM = bound picomolar. **a** Kd = 5.36×10^{-10} M, fmol/mg DNA = 237; **b** Kd = 47.6×10^{-10} M, fmol/mg DNA = 1394. White boxes are points not considered for the line fitting

cpm values of mibolerone uptake from the lowest (line 1) to the highest (line 9) single concentration of radioligand used, in the presence of TA or TA plus ORG2058 constant excess. Mibolerone binding showed minor variations in both soluble and nuclear fractions when comparing the excess addition of TA alone vs TA plus ORG2058, indicating negligible displacement of the tracer from other less specific steroid-binding sites.

With regard to the presence of different AR forms in human benign and malignant prostatic tissues, the ligand range and Kd cut-off values enabled us to distinguish type I (high affinity, low capacity) from type II (reduced affinity, higher capacity) binding sites. Scatchard plots from both ARc and ARn clearly show that type I and II receptors are generally represented by the first and second part of the competition curve, respectively (Figs. 1 and 2).

As shown in Table 2, type II AR was found to be present in both cell fractions of PCa (ARc 50%, ARn 75%) and BPH (ARc 59.4%, ARn 68.8%) tissues. Furthermore, the presence of nuclear type I AR was documented in over 90% of PCa tissues and BPH specimens. In contrast, soluble type I AR was revealed in a little bit more than 50% of both groups. Thus, only 50% of BPH and 46% of PCa tissues were positive for type I AR in both soluble and nuclear fractions, i.e., are likely to possess a "functional" receptor mechanism.

Figure 3 illustrates that, on the basis of a Kd cutoff value of 1.0 nM, the concentrations of type I binding sites in BPH were slightly higher for ARn than for ARc, but they never displayed high levels. On the other hand, type I AR content of soluble and nuclear fraction was almost equivalent in PCa samples.



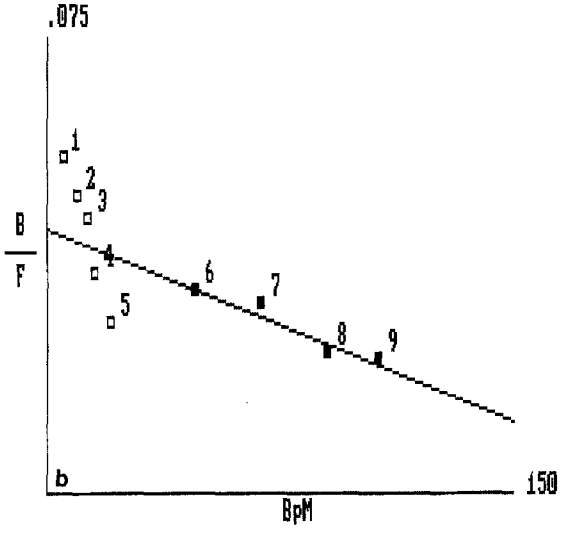


Fig. 2a, b. Scatchard plots of type 1 **(a)** and type II **(b)** androgen receptors in nuclear fraction of a prostate adenocarcinoma tissue. Nine points at various concentrations of radioligand mibolerone plus three competition points with excess cold mibolerone were used. B/F = bound over free; BpM = bound picomolar. **a** Kd = 5.51×10^{-10} M, fmol/mg DNA = 283; **(b)** Kd = 48.6×10^{-10} M, fmol/mg DNA = 1172. White boxes are points not considered for the line fitting

Table 3. Soluble fraction: femtomolar concentrations (fmol)[a] and dissociation constant (Kd)[b] median (range) values of type I and II androgen receptors (AR) in human benign prostate hyperplasia (BPH) and prostate cancer (PCa) tissues

	Type I AR		Type II AR	
	fmol	Kd	fmol	Kd
BPH	267* (35–1121)	5.36 (1.05–9.29)	2350 (276–30410)	33.5 (13.6–86.9)
PCa	566* (54–2106)	5.66 (1.01–9.21)	3950 (689–11135)	55.2 (15.2–91.4)

[a] Receptor concentrations are expressed as fmol/mg DNA
[b] Dissociation constant values are expressed as 1×10^{-10} M
* BPH vs PCa = $P < 0.04$, Wilcoxon test

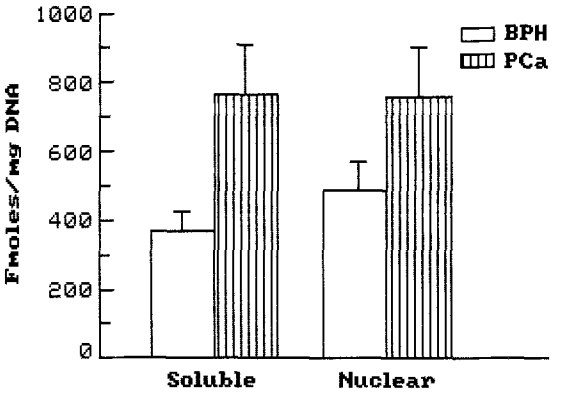


Fig. 3. Comparison of type I androgen receptor concentrations in soluble and nuclear fractions of 32 benign prostatic hyperplasia (BPH) and 24 prostate cancer (PCa) tissue specimens. Values are expressed as means ± SEM. For soluble fraction $P < 0.04$; for nuclear fraction $P < 0.003$ (two-tailed Student's t-test)

Table 4. Nuclear fraction: femtomolar concentrations (fmol)[a] and dissociation constant (Kd)[b] median (range) values of type I and II androgen receptors (AR) in human benign prostate hyperplasia (BPH) and prostate cancer (PCa) tissues

	Type I AR		Type II AR	
	fmol	Kd	fmol	Kd
BPH	410* (161–1474)	5.08 (2.17–9.30)	2121 (1116–6556)	50.0 (20.6–91.7)
PCa	711* (361–1109)	5.77 (1.82–8.75)	3039 (1605–5379)	40.1 (10.1–76.1)

[a] Receptor concentrations are expressed as fmol/mg DNA
[b] Dissociation constant values are expressed as 1×10^{-10} M
* BPH vs PCa = $P < 0.003$, Wilcoxon test

As shown in Tables 3 and 4, type II AR concentration values were higher (up to seven-fold for ARc and up to four-fold for ARn) than those of type I receptors in PCa tissues. In BPH they were up to eight- and five-fold higher than type 1 in soluble and nuclear compartments, respectively. Overall, Kd values ranged from 1.01 to 9.30 for type I and from 10.1 to 91.7×10^{-10} M for type II receptors.

As for comparison of BPH with PCa, type I AR concentrations were significantly higher in PCa than in BPH, for both soluble ($P < 0.04$) and nuclear ($P < 0.003$) fraction (Fig. 3, Tables 3 and 4). In contrast, no significant difference was found for type II AR content, although it was greater and much more scattered in soluble than in nuclear compartment of both PCa and, particularly, BPH tissues. However, the distribution of both type I and II receptors or, most important, the presence of type I binding sites in both cell fractions (i.e., a putative "functional" AR status), never showed statistically significant differences in PCa and BPH groups (Table 1), as clearly indicated by the range of respective P values (0.97 to 0.39; chi-square and Fisher's exact tests).

Discussion

In this study, the presence of a double binding site for steroid hormones, as already observed for both breast and endometrial tissues, has been clearly demonstrated in the human prostate, both in benign or malignant tissues. By definition, type I show high affinity and low capacity binding characteristics, while type II receptors have reduced affinity, but higher capacity for binding hormones [15].

The specificity and selectivity of androgen receptor proteins were also assessed by including both TA and ORG2058 in the assay prodecures; this may encompass criticism that might arise about a possible bias introduced by binding of the tracer to both glucocorticoid and progesterone receptors. Besides, it has been documented that incorporation of sodium molybdate in the homogenization buffer may augment the quantity of AR detected [39, 40]. The use of mibolerone in our studies has proved, therefore, to yield reproducible results.

We have shown that type I and II AR are present in both soluble and nuclear fractions of prostatic tissues. Our data also indicate that PCa tissues show a greater AR content than BPH in both cell compartments (Tables 3 and 4); this is in agreement with previous reports [41, 42]. However, it is noteworthy, in this study, that the difference was mainly confined to type I binding sites, whereas type II AR concentrations of BPH and PCa tissues did not differ significantly in either soluble and nuclear fractions.

Because nuclear type I AR is widespread in both BPH and PCa tissues, it is unlikely that it can accurately predict hormone sensitivity of tissues and clinical response of patients. In contrast, only 50% of BPH and 46% of PCa tissues were AR positive in the two cell fractions. Thus, the putative "integrity" of the receptor mechanism seems to be linked mainly to the presence of soluble type I receptors; this evidence fits well the hypothesis previously designed by Liao on the precise significance of cytoplasmic AR [43].

The true implication of type II binding sites in the receptor machinery is still obscure. The existence of double binding for steroid hormones may be interpreted as the expression of two distinct receptor entities or, alternatively, as the interconversion of two isoforms of the same receptor molecule.

To our knowledge, the presence of type I is usually but not always parallel to type II receptors, but not vice versa (data not shown). On the basis of this evidence, it is intriguing to speculate that type I and II receptors may play different roles or act in unity in hormonal regulation of cell growth and metabolism, depending on the cell type and especially the functional state of the cell.

Our study also provides a likely explanation for the variable results of AR determinations performed with classic biochemical methods or morphological visualization. For instance, unless appropriate ligand range and Kd cut-off values are carefully selected, the biochemical approach may lead to a mix-up of both AR types I and II. On the other hand, it is reasonable that gross morphological tests reveal predominantly type II AR [14], which is present in far greater amounts than type I.

Prospective studies are needed to establish whether the presence of type I and II receptors and their distribution within the cell play a functional role and can predict a response to hormone treatment in PCa patients. Such studies may also validate the hypothesis that only the presence of type I AR in soluble and nuclear cell fractions represents a "functional" receptor status for prostatic diseases, i.e., recognizes the hormone sensitiveness of tissues, in agreement with our previous findings obtained in breast and endometrial cancer.

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References

1. Huggins C, Hodges CV (1941) Studies on prostatic cancer. 1. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1:293
2. Hansson V, Tveter KJ, Attramadal A, Torgersen O (1971) Androgenic receptors in human benign nodular prostatic hyperplasia. Acta Endocrinol 68:79
3. Mobbs BG, Johnson IE, Connoly JG, Clark AF (1978) Androgen receptor assay in human benign and malignant prostatic tumor cytosol using protamine sulphate precipitation. J Steroid Biochem 9:289
4. Lieskovsky G, Bruchovsky N (1979) Assay of nuclear androgen receptor in human prostate. J Urol 121:54
5. Shain SA, Boesel RW, Lamm DL, Radwin HM (1980) Cytoplasmic and nuclear androgen receptor content of normal and neoplastic human prostates and lymph node metastases of human prostatic adenocarcinoma. J Clin Endocrinol Metab 50:704
6. Trachtenberg J, Bujnovszky P, Walsh PC (1982) Androgen receptor content of normal and hyperplastic human prostate. J Clin Endocrinol Metab 54:7
7. Ekman P (1982) The application of steroid receptor assay in human prostate cancer research and clinical management (review). Anticancer Res 2:163
8. Brendler CB, Isaacs JT, Follansbee AL, Walsh PC (1984) The use of multiple variables to predict response to endocrine therapy in carcinoma of the prostate: a preliminary report. J Urol 131:694
9. Fentie DD, Lakey WH, McBlain WA (1986) Applicability of nuclear androgen receptor quantification to human prostatic adenocarcinoma. J Urol 135:167
10. Kyprianou N, Williams H, Peeling WB, Davies P, Griffiths K (1986) Evaluation of biopsy techniques for androgen receptor assay in human prostatic tissue. Br J Urol 58:41
11. van Aubel OGJM, Bold-de Vries J, Blankenstein MA, ten Kate FJ, Schröder FH (1985) Nuclear androgen receptor content in biopsy specimens from histologically normal, hyperplastic and cancerous human prostatic tissue. Prostate 6:185
12. Ghanadian R, Auf G, Williams G, Davies A, Richards B (1981) Predicting the response of prostatic carcinoma to endocrine therapy. Lancet II:1418
13. Trachtenberg J, Walsh PC (1982) Correlation of prostatic nuclear androgen receptor content with duration of response and survival following hormonal therapy in advanced prostatic cancer. J Urol 127:466
14. Pertschuk LP, Eisenberg KB, Macchia RJ, Feldman JG (1985) Heterogeneity of steroid binding sites in prostatic carcinoma:

morphological demonstration and clinical implications. Prostate 6:35
15. Clark JH, Peck EJ Jr (1979) Female sex steroids receptors and function. In: Gross F et al. (eds) Monographs in endocrinology, Vol 14. Springer, Berlin Heidelberg New York
16. Lopes MTP, Liberato MH, Widman A, Brentani MM (1987) Occupied and unoccupied type II estrogen binding sites in human breast cancer. J Steroid Biochem 26:219
17. Turcotte G, Chapdelaine A, Roberts KD, Chevalier S (1988) Androgen binding as evidenced by a whole cell assay system using cultured canine prostatic epithelial cells. J Steroid Biochem 29:69
18. Chevalier S, Turcotte G, McKercher G, Boulanger P, Chapdelaine A (1990) Steroid metabolism and binding in relation to prostatic cell growth and differentiation in vitro. Ann NY Acad Sci 595:173
19. Laing LM, Smith DC, Calman KC, Smith MG, Leake RE (1977) Nuclear estrogen receptors and treatment of breast cancer. Lancet II:168
20. Thorsen T (1979) Occupied and unoccupied nuclear oestradiol and progesterone cytosol receptors. J Steroid Biochem 10:661
21. Leake RE, Habib F (1987) Steroid hormone receptors: assay and characterization. In: Green B, Leake RE (eds) Steroid hormones: a practical approach. IRL Press, Oxford, p 67
22. Castagnetta L, Lo Casto M, Mercadante T, Polito L, Cowan S, Leake RE (1983) Intratumoural variation of oestrogen receptor status in endometrial cancer. Br J Cancer 47:261
23. Castagnetta L, Traina A, Di Carlo A, Latteri AM, Carruba G, Leake RE (1987) Heterogeneity of soluble and nuclear oestrogen receptor status of involved nodes in relation to primary breast cancer. Eur J Cancer Clin Oncol 23:31
24. Leake RE, Laing L, Smith DC (1979) The role of oestrogen nuclear receptor measurements in the management of breast cancer. In: King RJB (ed) Steroid receptor assays in human breast cancer. Alpha Omega, Cardiff, p 73
25. Leake RE, Laing L, McArdle C, Smith DC (1981) Soluble and nuclear oestrogen receptor status in human breast cancer in relation to prognosis. Br J Cancer 43:67
26. Castagnetta L, Lo Casto M, Granata OM, Calabrò M, Ciaccio M, Leake RE (1987) Soluble and nuclear oestrogen receptors status of advanced endometrial cancer in relation to subsequent prognosis. Br J Cancer 55:543
27. Castagnetta L, Traina A, Di Carlo A, Carruba G, Lo Casto M, Mesiti M, Leake RE (1989) Do multiple oestrogen receptor assays give significant additional information for the management of breast cancer? Br J Cancer 59:636
28. Barnes DM, Skinner LG, Ribeiro GG (1979) Triple hormone-receptor assay: a more accurate predictive tool for the treatment of advanced breast cancer. Br J Cancer 40:682
29. Hahnel P, Partridge RR, Gavel L, Twaddle E, Rabdizal T (1980) Nuclear and cytoplasmic estrogen receptors and progesterone receptors in breast cancer. Eur J Cancer Clin Oncol 16:1027
30. Leake RE, Laing L, Calman KC, Macbeth FR, Crawford D, Smith DC (1981) Oestrogen receptor status and endocrine therapy of breast cancer: response rates and status stability. Br J Cancer 43:59
31. Castagnetta L, Carruba G, Calabrò M, Polito L, Blasi L, Pavone-Macaluso M (1991) Androgen receptor assays in specimens of prostatic tissue obtained by transurethral resection and transvesical adenomectomy. Urol Res 19:337
32. Crawford D, Cowan S, Hyder S, McMenamin M, Smith D, Leake RE (1984) A new storage procedure for tumor biopsies prior to estrogen receptor measurement. Cancer Res 44:2348
33. Schilling K, Liao S (1984) The use of radioactive 7alpha, 17alpha-dimethyl-19-nortestosterone (Mibolerone) in the assay of androgen receptors. Prostate 5:581
34. Jänne O, Kontula K, Vihko R (1976) Progestin receptors in human tissues: concentrations and binding kinetics. J Steroid Biochem 7:1061
35. Brinkman AO, Bolt J, van Steenbrugge GJ, Kuiper GG, Boer W de, Mulder E (1987) Characterization of androgen receptors in a transplantable human prostatic adenocarcinoma (PC-82). Prostate 10:133
36. Petra PH, Que BG, Namkung PC, Ross JB, Charbonneau H, Walsch KA, Griffin PR, Shabanowitz J, Hunt DF (1988) Affinity labeling, molecular cloning and comparative amino acid sequence analyses of sex steroid-binding protein of plasma. A multidisciplinary approach for understanding steroid-protein interaction and its physiological role. Ann NY Acad Sci 538:10
37. Lowry OH, Rosenbrough NJ, Farr AL, Randall J (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265
38. Burton KA (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315
39. Hawkins EF, Lieskrovsky G, Markland FS (1981) Molybdate and the measurement of androgen receptors in prostate cancer. J Clin Endocrinol Metab 53:456
40. Smith J, Chisholm GD, Habib FK (1983) Towards a reproducible method of estimating androgen receptors in human prostate. J Steroid Biochem 18:531
41. Benson RC Jr, Utz DC, Holicky E, Veneziale CM (1985) Androgen receptor binding activity in human prostate cancer. Cancer 55:382
42. Bowman SP, Barnes D, Blacklock NJ, Sullivan PJ (1986) Regional variation of cytosol androgen receptors throughout the diseased human prostate gland. Prostate 8:167
43. Hiipakka RA, Liao S (1984) Modulation of androgen receptor activity in the rat ventral prostate. Ann NY Acad Sci 438:54

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