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Animal models for hormone-dependent human breast cancer

Relationship between steroid receptor profiles in canine and feline mammary tumors and survival rate

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Summary. *The present study shows that canine and feline mammary tumors, like human breast tumors, can be polyreceptive, i.e., they can contain estrogen (ER), progestin (PR), androgen, glucocorticoid, and/or mineralocorticoid cytosol receptors. Furthermore, a follow-up of 45 bitches with mammary carcinoma has indicated that the survival rate is significantly higher in animals with receptor-rich (ER and/or PR) tumors. This indicates that these canine mammary tumors should be evaluated further for their suitability as an animal model for hormone-dependent human breast carcinoma.*

Introduction

It is now a well-established observation that patients with estrogen and, in particular, progestin receptor-positive malignant breast tumors have a higher survival rate and, in addition, are likely to respond favorably to hormone therapy [1, 6, 17, 25]. As a means of testing the efficacy of new drugs and treatment schedules, it would be very useful to have a good animal model with etiology and response characteristics similar to the human disease. In this respect, canine mammary tumors are intriguing: as in the human, mammary tumors in dogs are spontaneous (tumor material is readily available as the incidence of mammary tumors is three times higher in bitches than in women); the predominant malignant histological cell type is, as in humans, the adenocarcinoma; and the canine disease metastasizes similarly and evolves rapidly. Moreover, previous studies have shown that canine mammary tumors may contain cytosol estrogen (ER) and progestin (PR) receptors with an incidence comparable to that in humans [5, 18, 28]. A further report has established the presence of androgen receptors [8], but to our knowledge no studies of either glucocorticoid or mineralocorticoid cytosol receptors have been performed.

Mammary carcinoma is somewhat less frequent in cats than in dogs [9] but its histologic features are more similar to those of human carcinoma than are those of either murine or canine mammary carcinoma [23]. Although intact female cats have a seven-fold higher relative risk of developing mammary cancer than neutered females [9, 14], suggesting some involvement of steroid hormones, feline mammary carcinoma is generally regarded as a model for cancers less likely to respond to hormone manipulation [14, 23]. Estrogen receptor was detected in only two of 20 feline mammary carcinomas in one study [12] and in zero of 40 samples in another [23]. Two more recent studies [10, 16] have identified the presence of progestin receptors but not of either estrogen or androgen receptors.

In this paper, we present a more comprehensive analysis of steroid receptors in canine and feline mammary tumors, and we report that the canine tumors show greater polyreceptivity than the feline tumors. In addition, we provide evidence that in the bitch, as in humans, survival rate is enhanced in cases with receptor-rich tumors. This constitutes an argument for considering further canine mammary tumors as an animal model for human hormone-dependent breast cancer.

Materials and methods

Experimental animals. Receptor profiles were determined in 28 bitches of various breeds and 11 female cats (10 mongrels and 1 persian) with mammary tumors undergoing mastectomy at the Veterinary Clinic in Maisons-Alfort (France). Fractions of tumors were collected during surgery, trimmed of fat and connective tissue, and divided into two parts (5 : 1 ratio). The larger part was immediately frozen in liquid nitrogen for subsequent receptor assay within 4 weeks of removal; the remainder was stored in Bouin solution for histological examination according to the WHO classification [13].

Cytosol steroid receptor assays. The frozen tumor specimens were powdered in a high-pressure tissue pulverizer, weighed, and homogenized at low speed in 5 vol. (v/w) of ice-cold GTEM buffer (10 m*M* Tris-HCl, pH 7.6, containing 12 m*M* α-monothioglycerol, 1 m*M* EDTA, and 10% glycerol v/v) in a P10 Polytron homogenizer (three or four pulses of 4 s each). Cytosol was prepared by centrifuging the homogenates at 110,000 *g* for 60 min at 2° C.

Estrogen (ER), progestin (PR), androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) cytosol receptors were assayed according to published methods [20, 22, 26] as shown in Table 1[1]. Aliquots (0.1 ml) of cytosol (2 mg/ml) were incubated with increasing radioligand concentrations. Stabilization of the PR, GR, and MR receptors was achieved by adding 20 m*M* sodium molybdate to the incubation buffer. A

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1 Moxestrol (R 2858): 11β-methoxy-19-nor-17α-pregna-1,3,5(10) trien-20-yne-3,17β-diol; promegestone (R 5020): 17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione; metribolone (R 1881): 17β-hydroxy-17α-methyl-4,9,11-estratrien-3-one); RU 26988: 11β,17β-dihydroxy-17α-(1-propionyl) androsta-1,4,6-trien-3-one

Table 1. Steroid receptor assay methodology

Receptor	Radioligand[a]	Buffer[b]	Cold competitor	Incubation conditions
ER	(11-methoxy-^{3}H)-Moxestrol s.a. ~ 85 Ci/mmol	GTEM	–	5 h at 25° C and overnight at 0° C
PR	(17α-methyl-^{3}H)-Promegestone s.a. ~ 85 Ci/mmol	GTEM + 20 m*M* sodium molybdate	500 excess cortisol	Overnight at 0° C
AR	(17α-methyl-^{3}H)-Metribolone s.a. ~ 85 Ci/mmol	GTEM	500 excess triamcinolone acetonide	Overnight at 10° C
GR	(6,7-^{3}H)-Dexamethasone s.a. 30–50 Ci/mmol	GTEM + 20 m*M* sodium molybdate	–	3 h at 20° C and overnight at 0° C
MR	(1,2,6,7-^{3}H)-Aldosterone s.a. 80–105 Ci/mmol	GTEM + 20 m*M* sodium molybdate	200 excess RU 26988	3 h at 20° C and overnight at 0° C

[a] All radioligands were purchased from New England Nuclear (Boston, Mass.)
[b] GTEM = 10 m*M* Tris-HCl pH 7.6, containing 12 m*M* α-monothioglycerol, 1 m*M* EDTA, and 10% glycerol v/v

500-fold excess of triamcinolone acetonide was added to the (^{3}H)-metribolone to ensure that only androgen binding was measured [27] and a 200-fold excess of a pure glucocorticoid, RU 26988, to the (^{3}H)-aldosterone to eliminate any interference from GR binding in the measurement of MR [22, 27]. The buffers did not contain protease inhibitors although recent experience from our laboratory has suggested that the proteolytic activity in canine mammary tumor cytosol is greater than in human breast tumor cytosol. Although none of the radioligands used binds with high affinity to specific plasma proteins such as corticosteroid and testosterone binding globulins [26], which could contaminate dog and cat cytosol preparations [7, 11, 30, 32], the possibility of residual plasma binding was eliminated by adding a 500-fold excess of cortisol to the PR buffer and of estrone to the AR buffer. Non-specific binding was evaluated by the addition of a 100-fold excess of the corresponding non-radiolabeled ligand.

The incubated cytosol was stirred for 25 min at 4° C with 0.5 ml Dextran-coated charcoal (DCC) suspension (0.5% charcoal Norit A (Sigma), 0.05% Dextran T.70 (Pharmacia) plus 0.28% gelatin for cytosols containing < 1 mg/ml protein in GTEM buffer, and centrifuged for 10 min at 300 *g*. Bound labeled steroid was determined by counting the radioactivity in an aliquot of supernatant, and the concentration of specific binding sites was estimated from a Scatchard plot. The amount of cytosol available was not always sufficient to perform all five receptor assays in triplicate. In some instances, therefore, GR and MR were not assayed.

Survival rate. Survival rate was estimated in a series of 45 untreated dogs with malignant tumors which had been previously tested for ER and PR [28]. A cut-off value of 10 fmol/mg protein was used to divide tumors into receptor-rich and receptor-poor categories by analogy with human tumors.

Results

Tumor polyreceptivity

Table 2 gives the receptor concentrations recorded in 19 malignant and nine benign canine mammary tumors. Since a previous study [28] has shown that no significant difference is recorded in the incidence of estrogen (ER) and progestin (PR) receptors according to breed, age, and number of pregnancies or pseudopregnancies, no information relating to the clinical history of the bitches is given. The results confirm the presence of ER and PR, although this incidence was lower than that previously recorded [5, 28]. Only 11 out of a total of 28 tumors (39%) were either ER$^+$ and/or PR$^+$ on the basis of a cut-off value of 10 fmol/mg protein. This discrepancy with previous results (65%) may be explained by the size of the experimental sample, which was too small to be truly representative of the overall population. However, if only adenocarcinomas, the most frequent malignant tumor, are taken into consideration, the rate of ER and/or PR positivity recorded here (7 of 13 = 54%) corroborates previous data (58%). Malignant tumors in fact represented 65% of the overall sample, and two-thirds of these were adenocarcinomas, which constituted the most receptor-rich category. None of the four solid carcinomas nor the fibrosarcoma was receptor-positive. Among the benign tumors, one adenoma, one mixed tumor, and the single case of lobular hyperplasia were receptor-positive.

Our results also support previous evidence [8] for the presence of a cytosol androgen receptor in some tumors. The incidence was low, as in humans [2, 3, 31]. Only four of 28 tumors could be considered AR$^+$. The AR values tended to be highest in tumors with high ER and PR values.

In cases (14 of 28) where enough cytosol was available to perform simultaneous corticoid receptor assays, it was noted that except in one instance (B67), the GR and MR levels recorded were at the limit of detection and probably of minor significance considering the imprecision of the assay technique in this low concentration range.

Table 3 gives the results obtained for feline mammary tumor cytosol. These show the presence of ER and/or PR in substantial amounts in two malignant tumors (one adenocarcinoma and one solid carcinoma) and in two benign tumors (one adenoma and one lobular hyperplasia). Surprisingly, the highest PR value (tumor no. 64) was found in an adenocarcinoma containing no detectable cytosolic ER. This is in agreement with the results of Johnston et al. [16], who report that none of the seven cytosols they tested had detectable ER,

Table 2. Receptor profiles in mammary tumors of dogs

		Receptor concentration (fmol/mg protein)				
		ER	PR	AR	GR	MR
Malignant tumors (n = 19)						
B 46	Adenocarcinoma[a]	3	1	6	3	3
B 53		5	6	6	3	3
B 54		38	22	13	1	1
B 66		4	5	5	ND	ND
B 70		4	3	9	ND	ND
B 73		23	35	18	ND	ND
B 47	Adenocarcinoma[b]	14	18	13	ND	ND
B 59		21	30	8	1	1
B 62		3	6	3	2	2
B 64		3	3	5	ND	ND
B 67		14	2	5	12	12
B 77		2	10	3	ND	ND
B 48	Adenocarcinoma[c]	10	7	2	ND	ND
B 50	Solid carcinoma	5	6	4	2	2
B 52		0	1	1	0	0
B 60		2	2	3	2	2
B 65		0	6	2	2	2
B 61	Fibrosarcoma	0	0	0	0	0
B 85	Carcinosarcoma	21	5	7	ND	ND
Benign tumors (n = 9)						
B 56	Adenoma	8	11	14	2	2
B 80		4	8	4	ND	ND
B 55	Benign mixed tumor	5	2	2	2	2
B 57		9	7	3	1	1
B 58		8	17	9	ND	ND
B 69		3	3	3	ND	ND
B 82		9	5	4	ND	ND
B 83		9	7	5	ND	ND
B 79	Lobular hyperplasia	15	14	7	ND	ND

ER, estrogen receptor; PR, progestin receptor; AR, androgen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor

ND, not determined on account of limited amount of cytosol available

[a] Tubular, simple type

[b] Tubular, complex type

[c] Papillary, simple type

Table 3. Receptor profiles in mammary tumors of cats

		Receptor concentration (fmol/mg protein)		
		ER	PR	AR
Malignant tumors (n = 9)				
B 18	Adenocarcinoma (tubular, simple type)	1	4	ND
B 21		1	0	ND
B 51		3	0.5	26
64		0	28	ND
B 74		0.5	4	10
B 76		4.5	1.5	13
B 86		1.5	3	9
B 44	Solid carcinoma	3	0.5	ND
B 78		14	0	7
Benign tumors and dysplasia (n = 2)				
B 81	Adenoma	31	18	13
B 87	Lobular hyperplasia	5	26	3

ND, not determined on account of limited amount of cytosol available

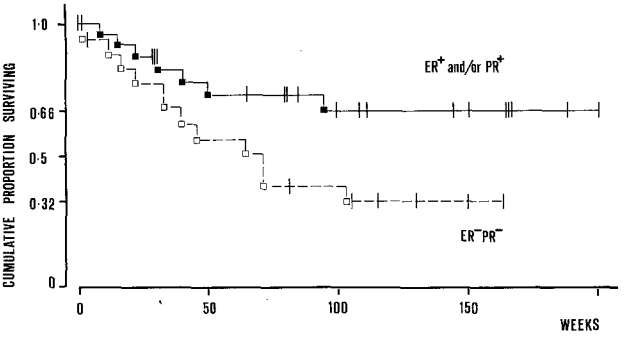

Fig. 1. Kaplan-Meier survival curves of bitches undergoing mastectomy for mammary cancer according to ER and/or PR status of the tumor (χ^2 = 4.18, $P < 0.05$ in log rank test)

whereas all contained PR. These authors suggest that feline mammary adenocarcinoma may be representative of the small group of ER^-PR^+ human mammary tumors. However, the case may be otherwise for solid carcinomas (tumor B78 was ER^+PR^-) and for benign tumors and dysplasia, which contained some ER.

The hormonal pathogenesis (hyperprogesteronism) of benign fibroglandular proliferations of the mammary gland in the cat has already been reported; these growths regress following ovariectomy [4, 15]. Three adenocarcinomas, all ER^-PR^-, were nevertheless AR^+, a result rather different from that recorded in dogs. The ER^+PR^+ benign tumor was also AR^+. In only one instance (B51) could corticoid receptors be assayed. This tumor, which was rich in AR but low in ER and PR, was found to contain equivalent and significant amounts of both GR and MR (6 fmol/mg protein).

Survival rate

The 45 bitches were divided into two categories: those with ER and/or PR levels of 10 fmol/mg protein or above (25 bitches) and those with low receptor levels (20 bitches). A comparison of survival rates (Fig. 1) shows that according to a log rank test, survival was significantly higher in the receptor-rich category (χ^2 = 4.18, $P < 0.05$).

Discussion

Chemically induced (e.g., DMBA-induced) animal tumors or viral neoplasms of inbred strains of rodents are generally non-metastatic and thus on the whole constitute unsatisfactory models for human breast carcinoma, which is spontaneous and highly metastatic. By contrast, canine mammary tumors resemble those in man insofar as they are also spontaneous (although three times more prevalent [9]) and metastatic; also, they have certain histological similarities [24] and they show an analogous incidence of steroid hormone receptor positivity [28]. The present study develops the analogy between canine and human mammary tumors further by establishing in canine tumors a low but apparently significant incidence of androgen,

mineralocorticoid and glucocorticoid receptors, as is also recorded in humans [2, 3, 22, 31], and by clearly demonstrating a higher survival rate with receptor-rich tumors, as is known to be the case in humans [1, 6, 17, 19, 25]. Thus, in dogs and in humans, the assay of ER and particularly of PR [19] constitutes a rapid and simple method of establishing a prognosis, since their presence is related to the degree of differentiation of the tumor and its histoprognostic grade regardless of histological type [21]. Together with the assessment of lesional elastosis in humans [29], steroid receptor assays on tumor biopsies constitute an indicator of disease-free interval [19].

In humans, the presence of ER and/or PR is, in addition, a positive indicator for a favorable response to hormonal therapy and is used in patient selection. It may be that in the dog, too, hormonal therapy is most effective in animals with ER^+ and/or PR^+ tumors. If this proves to be the case, the canine mammary tumor might then be considered a good model for the human disease and might be used in the systematic development of new endocrine therapies that may be effective in the human.

In feline mammary carcinoma, which in its histologic features is more similar to human carcinoma than is canine carcinoma [23], the incidence of ER and PR seemed to be very low. The association of this observation with the poor prognosis of mammary carcinoma in the cat [23, 33] is very similar to the situation in the human when receptor levels are low [19]. A relatively high proportion of cat tumors contained androgen receptor. Should this incidence be confirmed in a larger sample reflecting the overall population, it would be interesting to investigate whether the progression of feline mammary cancer might be successfully controlled by anti-androgen therapy.

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