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RELATIONSHIP BETWEEN TUMOUR AROMATASE ACTIVITY, TUMOUR CHARACTERISTICS AND RESPONSE TO THERAPY

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Summary—Aromatase activity has been measured in human breast cancers by incubating tumour minces with [7α-^{3}H]testosterone and characterizing purified oestradiol (E_2) fractions by chemical derivative formation. Of 247 primary tumours, 178 showed evidence of oestrogen biosynthesis, levels varying between 0.5 and 12.5 fmol E_2 produced/h/g tissue. These values were quantitatively small but at least comparable with those in other peripheral tissues. There was no correlation between presence or level of aromatase activity and the histopathology of the tumours although oestrogen biosynthesis was more likely to be present in more cellular tumours. Aromatase activity was also unrelated to age, menopausal status, lymph node status and T stage of the patient from which the tumour was derived. In a subgroup of patients presenting without clinical evidence of distant metastatic disease, no significant relation was detected between tumour aromatase and disease-free interval, but tumours without aromatase activity were associated with increased survival at 36 months after primary treatment. A statistically significant correlation was also detected between the presence of tumour aromatase and oestrogen receptors. Furthermore, in small subgroups of patients with "advanced" breast cancer tumour aromatase was related to response to aminoglutethimide but not tamoxifen therapy. Whilst these results do not conclusively define a role for local synthesis of oestrogen in the progression of breast cancer, this possibility still exists and further studies on tumour aromatase are warranted.

INTRODUCTION

Certain breast cancers appear to require oestrogen for their continued growth [1]. In premenopausal women this hormone requirement seems to be largely satisfied by secretion from the ovary [2]. However, in postmenopausal women, the principle sites of oestrogen biosynthesis are peripheral tissues. These include skin [3], muscle [4], adipose tissue [4, 5] and breast cancers themselves [6]. The potential for oestrogen biosynthesis or aromatase activity in breast cancers is the subject of this review which seeks to assess the significance of such local biosynthesis by investigation of putative relationships with patient and tumour characteristics.

MATERIALS AND METHODS

Histologically proven breast cancer was obtained from primary tumour in 247 patients, invaded axillary lymph node in 15 women and skin metastasis in two cases. Both primary tumour and lymph node were obtained from five individuals. Following surgical excision, specimens were immediately placed on ice and transferred to the laboratory. Tumour was dissected free of fat and portions (500 mg) finely sliced and incubated for 2 h at 37°C in Krebs Ringer phosphate buffer pH 7.4 containing a NADPH generating system and [7α-^{3}H]testosterone (22.5 μCi). The reaction was stopped by addition of methanol (final concentration 80%) and the incubates were stored at −10°C until being processed further. Before extraction, radioinert oestradiol (500 μg) was added to enable monitoring of procedural losses. Metabolites were then extracted and purified by thin-layer chromatography as described previously [7, 8]. Oestrogen fractions were characterized by chemical derivative formation (acetates and methyl ethers), purity being based on: (a) similar chromatographic mobility of parent steroids and derivatives to authentic standards; and (b) consistency of specific radioactivity between parent steroid and

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derivatives during these procedures. Using these techniques it is difficult to detect conversions below 0.02% of the original precursor unless the above criteria are relaxed. For the purposes of this paper, therefore, conversions $\geqslant 0.02\%$ have been considered as evidence of potential for aromatization.

The criteria for histopathology and grading were as previously published [9] and the method for measurement of oestrogen receptors as described by Hawkins *et al* [10].

RESULTS

Incidence and level of aromatase

Of the 247 primary breast cancers investigated, 178 (72%) showed evidence of aromatase activity (Table 1). Additionally in 8 of 15 (53%) invaded lymph nodes and both skin metastasis possessed the activity. Levels of conversion to oestrogen varied from 0.02 to 0.83% (median conversion 0.08%) and were not significantly different between primary tumours and lymph nodes. (Skin metastases both showed relatively high levels of biosynthesis, i.e. 0.12 and 0.14%.) In five women it was possible to obtain both primary tumour and lymph node. These results are presented in Table 2 and show that whilst there were minor quantitative differences between the two sources of tissue, aromatase activity was of a similar magnitude in primary tumour and lymph node. Furthermore the two primary tumours without aromatase were associated with lymph nodes also devoid of activity.

Relationship between aromatase and tumour histology

Histological assessment for cellularity, grading and special features was performed on sections from 227 of the primary tumours by one of us (TJA) without previous knowledge of the metabolism results. As is shown in Table 3, tumours categorized as being of either low, moderate or high cellularity were subdivided accordingly to whether aromatase activity was absent, present in low (arbitrarily $<0.1\%$ conversion to oestradiol) or high levels ($\geqslant 0.1\%$ conversion). It can be seen that tumours without aromatase activity were mostly of low and moderate cellularity whereas those with high activity predominantly had high cellularity. This trend for aromatase activity to be positively associated with tumour cellularity was statistically significant ($P < 0.001$). It should also be emphasized, however, that 15% of tumours without evidence of aromatase activity were of high cellularity and conversely 17% of cancers displayed high aromatase activity in spite of their low cellularity.

No significant correlations were observed, qualitatively or quantitatively between aromatase and either histological subtype, tumour grade or special features such as lymphocytic infiltration or necrosis (data not shown).

Table 1. Incidence of aromatase in different sources of tumour tissue

	Total	With aromatase
Primary	247	178 (72%)
Lymph node	15	8 (53%)
Skin metastasis	2	2 (100%)

Table 2. Aromatase activity in primary tumour and involved lymph node from the same patients

Patient	A	B	C	D	E
Primary	0.027	0.110	0.068	<0.02	<0.02
Lymph node	0.041	0.080	0.065	<0.02	<0.02

Values are % conversion of 7α[^{3}H]testosterone to oestradiol after *in vitro* incubation.

Tumour aromatase and patient characteristics

No significant qualitative or quantitative relationship was apparent between tumour aromatase and age of the patient, menopausal status or stage of disease as monitored by lymph node involvement or tumour clinical size (data not shown).

Tumour aromatase and prognosis in patients free of distant metastatic disease

Three-year follow-up data were available on a subgroup of 131 patients who presented clinically without distant metastatic disease and were treated by local breast surgery. The relationships between recurrence of disease and survival at 36 months and aromatase in the primary tumour are shown in Tables 4a and b. No significant correlation was apparent between tumour aromatase status and recurrence by 36 months. However, patients having tumours without aromatase activity were associated with increased likelihood of overall survival at 3 yr compared with those with aromatase-positive tumours.

Table 3. The relationship between tumour cellularity and aromatase activity

	Cellularity		
	Low	Moderate	High
Aromatase			
None	24 (35%)	34 (50%)	10 (15%) (68)
Low	22 (19%)	55 (49%)	36 (32%) (113)
High	8 (17%)	14 (31%)	24 (52%) (46)

Kendall rank coefficient = 0.25, $P < 0.001$.

Table 4. The relationship between tumour aromatase and (a) recurrence at 3 yr and (b) survival at 3 yr

	(a) Recurrence		(b) Survival	
	Disease-free	Recurrence	Alive	Dead
Aromatase				
+ve	55	33	67	21
−ve	28	15	40	3
	$P = 0.93$		$P = 0.028$	

P-values by Fisher's exact text.

Relationship between tumour aromatase, oestrogen receptors and response to endocrine therapy

The relationship between aromatase and tumour oestrogen receptors in 247 of the tumours studied is presented in Table 5. All possible combinations of the two parameters were possible in different subgroups of tumours. However aromatase-positive tumours were mostly oestrogen receptor-positive whereas those without aromatase were more evenly distributed between the receptor-positive and -negative populations. This positive association between the presence of oestrogen receptors and aromatase was statistically significant.

Twenty-three of the subjects in the study were postmenopausal patients with advanced oestrogen receptor-positive breast cancer who were treated with aminoglutethimide (1 g daily) and hydrocortisone (40 mg daily). Response to therapy could therefore be related to tumour aromatase activity as is shown in Table 6. All five tumours without aromatase activity failed aminoglutethimide therapy whereas 11 of 18 tumours with oestrogen biosynthesis responded to the treatment. This positive relationship between tumour aromatase and response to aminoglutethimide was statistically significant.

A further 24 postmenopausal patients had oestrogen receptor-positive advanced disease and were treated with tamoxifen. The relationship between response to tamoxifen and aromatase status is shown in Table 7. Although tumours with aromatase activity were more likely to respond to tamoxifen, no significant correlation was apparent between tumour aromatase and response to antioestrogen.

Table 5. The relationship between tumour aromatase and oestrogen receptor status

	Oestrogen receptors	
	+ve	−ve
Aromatase		
+ve	131	35
−ve	50	31

$\chi^2 = 8.21$, $P < 0.01$.
Oestrogen receptor +ve tumours contain >5 fmol receptor/mg cytosol protein.

Table 6. The relationship between tumour aromatase and clinical response to aminoglutethimide-hydrocortisone

	Clinical response	
	Partial	Static/progressive
Aromatase		
+ve	11	7
−ve	0	5

$P = 0.047$.
P-value by Fisher's exact test.

DISCUSSION

Using a definitive method by which to characterize oestrogen production, aromatase activity has been detected in over 70% of *in vitro* incubations of breast cancers. The levels of conversion of androgen to oestrogen were small (0.02–0.83%) but comparable or greater than those detected in other peripheral tissues [11, 12]. Furthermore, if this level of activity was reflected *in vivo*, it would represent pmol amounts of oestrogen being produced on site within tumour tissue; these levels being sufficient to elicit oestrogenic responses [13], including growth of oestrogen-sensitive cells [14].

In an effort to determine whether local oestrogen biosynthesis within breast cancers may be of clinical significance, patient and tumour parameters which might potentially influence aromatase activity have been investigated. Aromatase activity could be detected in different sources of tumour material, viz. primary tumour, invaded axillary lymph node and skin metastasis. Although the incidence of aromatase was lower in lymph nodes than in primary tumours, the difference was not statistically significant. Furthermore, in a limited series of individuals from whom both primary and lymph node were available for study, the qualitative pattern of aromatase activity was identical and level of activity was similar in the two types of tissue. There is therefore little to suggest that with metastatic spread or advancement of disease, tumour aromatase status changes. Although two skin metastasis showed relatively high levels of oestrogen biosynthesis, the observation can only be anecdotal because of sample size.

Table 7. The relationship between tumour aromatase and clinical response to tamoxifen

	Clinical response	
	Partial	Static/progressive
Aromatase		
+ve	9	4
−ve	5	6

$P = 0.45$.
P-value by Fisher's exact text.

The statistically significant positive correlation between tumour aromatase and cellularity strongly suggests that cancer cells are the principle source of aromatase activity in the tumour biopsies. Thus it was possible to detect aromatase activity in 60 of 70 tumours with high cellularity. Conversely the inability to detect aromatase activity in certain tumours may be due to their low cellularity, 85% of tumours without detectable aromatase activity being of low or moderate cellularity. Although aromatase activity has been detected in nonmalignant components of the breast [15, 16], level of activity is invariably lower than in breast cancers [12] and oestrogen biosynthesis has never been convincingly shown to be present in normal parenchyma [11, 17, 18]. Nevertheless, since about 15% of low cellularity tumours displayed high levels of aromatase and conversely a similar percentage of high cellularity tumours showed no activity, it is clear that factors in addition to cellularity influence oestrogen biosynthesis within breast tumours. In this respect other pathological characteristics seem to have relatively minimal effects, no significant correlation being detected between aromatase activity and pathological grade, special pathological subtypes and features such as necrosis and lymphocytic infiltration.

Although aromatase in other peripheral tissues seems to increase with age [19], no such relationship was found between tumour oestrogen biosynthesis in the present study. Menopausal status also failed to affect the incidence or levels of tumour activity. The lack of an association with lymph node involvement or tumour size would suggest that tumour aromatase is not of prognostic significance, a conclusion supported by the observation that there was no significant difference in tumour aromatase between patients presenting with recurrence within 36 months and those remaining disease-free at this time. In contrast, however, tumours without aromatase seem to confer significantly increased likelihood of survival at 36 months, only three patients of the subgroup of 43 aromatase-negative tumours from women presenting without distant metastasis having died within this time period. Since aromatase-negative tumours have a similar rate of recurrence at 36 months but a significantly improved survival at this time compared with aromatase-positive tumours it would seem that tumours without aromatase are associated with an extended time interval between recurrence and death. However, 36 months is a comparatively short time of follow-up and further study is needed to confirm this suggestion.

Controversy exists within the published literature as to the potential relationship between tumour aromatase and oestrogen receptor status [11, 20, 21]. The present study found a significant trend for tumours possessing aromatase activity to be oestrogen-receptor positive. However it is clear that aromatase activity may be found in both oestrogen receptor-positive and receptor negative tumours. Conversely oestrogen receptor-positive cancers may or may not synthesize oestrogen. The clinical relevance of oestrogen biosynthesis in oestrogen receptor-negative tumours is debatable as such tumours rarely respond to antioestrogenic measures [22]. If, therefore, tumour oestrogen biosynthesis is of significance it seems more likely to be in oestrogen receptor-positive tumours in which a mechanism exists to process the synthesized steroid. The hypothesis may be put forward that tumours which not only possess oestrogen receptors but synthesize oestrogen are more likely than tumours with oestrogen receptors alone, to respond to measures which block biosynthesis. The results presented in this study relating response to aminoglutethimide with tumour aromatase would support such a concept. Thus whilst not all tumours with aromatase activity responded to therapy, the aromatase inhibitor was more likely to produce beneficial effects in this group and no tumour without aromatase activity responded to treatment. It therefore seems logical to treat tumours possessing both oestrogen receptors and aromatase activity with some form of anti-aromatase regime. Interestingly, the relationship between tumour aromatase and clinical response to tamoxifen did not reach statistical significance (although the number of patients studied was small and a trend was evident) which suggests that tumour aromatase has a particular association with response to aminoglutethimide and is not a simple marker of endocrine sensitivity.

In conclusion aromatase activity has been detected in the majority of breast cancers at levels which are small but comparable with other peripheral tissues. The close association between activity and tumour cellularity suggests that oestrogen biosynthesis is predominantly associated with malignant cells. However no conclusive data have been found to relate aromatase activity with other histological features, progression or natural history of breast

cancer. Nevertheless the significant associations between: (i) lack of tumour aromatase and increased survival at 36 months in patients without clinical evidence of widely disseminated breast cancer; and (ii) presence of tumour aromatase and response to aminoglutethimide in patients with advanced breast cancer suggest that local oestrogen biosynthesis promotes tumour growth in certain patients. This warrants further investigation.

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