Prognostic Value of Ornithine Decarboxylase and Polyamines in Human Breast Cancer: Correlation with Clinicopathologic Parameters

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ABSTRACT

The polyamines putrescine, spermidine, and spermine and ornithine decarboxylase (ODC), the rate-limiting enzyme in their biosynthetic pathway, play an important role in cell proliferation, differentiation, and transformation. In the present study, we have analyzed polyamine concentrations and ODC activity in samples from benign breast diseases ($n = 36$), benign breast tissue adjacent to the primary carcinoma ($n = 19$), and breast carcinoma ($n = 104$). ODC activity in primary carcinoma was significantly higher (2.42 ± 0.22 nmol CO_2/h g; $P < 0.001$) than that found in benign breast (0.62 ± 0.15 nmol CO_2/h g) or in breast tissue adjacent to the primary carcinoma (0.52 ± 0.16 nmol CO_2/h g). The total polyamine content of breast cancer tissues was higher than in benign breast diseases (704.3 ± 38.3 nmol/g wet weight *versus* 295.8 ± 27.4 nmol/g wet weight) and correlated well with ODC activity (Pearson, $r = 0.42$; $P < 0.001$). ODC activity correlated with histological grade, peritumoral lymphatic or blood vessel invasion, S-phase fraction, and cathepsin D. Total polyamine concentration increased with S-phase fraction, cathepsin D, and aneuploidy. No significant correlation was found between ODC or polyamines and tumor size, lymph node involvement, or steroid receptor status. A major finding in our study was that ODC activity was an independent prognostic factor for recurrence and death. The results indicate that the estimation of ODC activity and polyamines in human breast carcinoma might be useful to determine tumor aggressiveness and suggest that ODC may have a potential value as both a prognostic factor and a chemoprevention target in human breast cancer.

INTRODUCTION

The polyamines (PUT[2], SPD, and SPN) comprise a family of aliphatic cations that occur ubiquitously in nature (1). They are critical for cell proliferation, differentiation and transformation, and are involved in DNA, RNA, and protein synthesis, as well as in stabilizing membrane and cytoskeletal structures (2–4). ODC (EC 4.1.1.17) is a key enzyme in polyamine biosynthesis, catalyzing the conversion of L-ornithine into PUT. It is found in very limited amounts in quiescent cell, although its activity rapidly and markedly increases in response to many trophic stimuli (hormones, growth factors, and tumor promoters) and during tissue regeneration (5).

Many different studies from animal models have shown that polyamines accumulate in cancer cells and that the use of inhibitors of polyamine biosynthesis or polyamine analogues has a remarkable potential to block tumor growth and prevent metastases (6, 7). The molecular mechanisms by which the manipulation of polyamine levels affect cancer cell growth remain to be established, although several studies with breast cancer cells have suggested an interesting interrelation between estrogens and growth factors and polyamines (8–15). Few studies have clearly shown that human malignant breast tissues contain larger amounts of polyamines than normal ones (16, 17). Besides, several studies have also suggested a possible role of polyamine metabolism in regulating oncogene expression and function (18, 19). Recently, it has been postulated that the *ODC* gene may act as an oncogene because the overexpression of this gene is essential for cell transformation (20, 21). Overproduction of ODC by stimulating the translation of its mRNA seems to be also critical in neoplastic transformation (22). Overexpression of ODC may also lead to increased tumor invasiveness and angiogenesis (23, 24). High expression of some oncogenes, as well as deletion of many chromosome loci associated with putative suppressor oncogenes in breast cancer, have been reported (25, 26), and several oncogenes have been shown to have independent prognostic value (27).

The development of new methods to obtain prognostic information presents increasing interest in the treatment of breast cancer (28). In the past decade, it was reported that ODC was not detectable in normal breast or in benign mammary disease tissues, whereas breast carcinomas with higher ODC activity had higher cellularity and nuclear anaplasia and lower histological differentiation than those in which ODC activity

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[2] The abbreviations used are: PUT, putrescine; SPD, spermidine; SPN, spermine; ODC, ornithine decarboxylase; PLBI, peritumoral lymphatic or blood vessel invasion; DFMO, difluoromethyl-ornithine; SPF, S-phase fraction; ER, estrogen receptor; PR, progesterone receptor; HG, histological grade.

was not detectable (29). Although it was recently postulated that increased ODC is a negative prognostic factor for disease-free survival and overall survival in breast cancer patients (30), the analysis of a larger number of patients would be required to confirm this claim. Moreover, ODC activity and polyamines have not been evaluated simultaneously in specimens from human breast cancer and correlated with other histopathological or biochemical markers of malignancy. In the present study, ODC activity, polyamines, cathepsin D, and steroid receptors have been analyzed in benign mammary disease tissues and in breast carcinomas to evaluate combined relationships between ODC/polyamines with clinical and histopathological indicators of tumor aggressiveness, as well as the predictive value of ODC/polyamines in human breast cancer.

PATIENTS AND METHODS

Patients. Thirty-six patients with benign breast diseases (including 21 fibroadenomas and 15 fibrocystic diseases) were investigated as the control group of nonmalignant disorders (median age, 32 years; range, 18–41 years). A total of 104 patients with breast cancer were included in this study (median age, 57 years; range, 28–75 years). All primary breast tumor specimens were obtained from patients who underwent surgical operation at Virgen de la Arrixaca Hospital (Murcia, Spain), from January 1990 to February 1993. The patients selected met the following criteria: primary unilateral breast carcinoma, operable with no clinical metastasis; no radiation therapy or chemotherapy before surgery; complete information of clinical, histological, and biological data; and having specimens with sufficient tumor material remaining for assaying enzymes and polyamines. The distribution of clinical and pathological data for the entire patient population is listed in Table 1. Eighty-nine patients had undergone a modified radical mastectomy, and 15 patients had a quadrantectomy plus radiotherapy. Sixty-three patients had received postoperative radiation therapy as part of their locoregional treatment. Eighty-seven patients had received adjuvant therapy, consisting of hormone therapy (tamoxifen, $n = 28$), chemotherapy (mainly a combination of six cycles of cyclophosphamide, methotrexate, and 5-fluorouracil, $n = 40$), or both ($n = 19$).

After surgery, all patients underwent clinical, radiological, and biological examinations every 3 months for the first 2 years and annually thereafter. The median follow-up was 63 months (maximum, 92 months). At 5 years, 55 patients had relapsed (7 had local recurrences, 48 had distant metastases, and 4 had both) and 29 of 55 patients had died of breast cancer.

Table 1 Characteristics of the patients

No. of patients	104
Median age (years)	56
Menopausal status	
Pre/perimenopausal	29 (27.9%)
Postmenopausal	75 (72.1%)
Histology type	
Ductal	89 (85.6%)
Lobular	6 (5.8%)
Other histology[a]	9 (8.6%)
Tumor size (pT, mm)	
T_1 (<20)	17 (16.3%)
T_2 (20–50)	66 (63.5%)
T_3 (>50)	21 (20.2%)
Nodal status (pN)	
Negative	40 (38.5%)
Positive	64 (61.5%)
H Grade[b]	
G I	13 (12.5%)
G II	68 (65.4%)
G III	23 (22.1%)
PLBI	
Negative	75 (72.1%)
Positive	29 (27.9%)
Necrosis	
Negative	62 (59.6%)
Positive	42 (40.4%)
Steroid receptor status	
ER−, PR−	31 (29.8%)
ER−, PR+[c]	3 (2.9%)
ER+, PR−	21 (20.2%)
ER+, PR+[c]	49 (47.1%)
DNA ploidy	
Diploid	50 (52.6%)
Aneuploid	45 (47.4%)
SPF	
<10%	49 (51.6%)
≥10%	46 (48.4%)

[a] Other histology: five medullaries, two colloids, and two papillaries.
[b] By the method of Scarff, Bloom, and Richardson.
[c] Positive if ≥10 fmol/mg protein.

MATERIALS AND METHODS

The macroscopic tumor size, number of positive axillary nodes, and steroid receptor status were established at the time of surgery. Tumor stages were defined according to the WHO classification (31), and the HG was defined according to Bloom and Richardson (32). The presence of tumor emboli in endothelial-lined channels was designated as PLBI. A representative tumor section was obtained fresh from the operating room, handled on ice, and stored in liquid nitrogen within 15 min for routine steroid hormone receptor assay, enzymatic determinations, and polyamine analysis. A portion of the tumor tissue was homogenized in a microdismembrator (Polytron CH-610; Kriens-Luzen, Switzerland) and suspended in standard receptor buffer [10 mM phosphate (pH 7.4), 1.5 mM EDTA, 5 mM DTT, 5 mM sodium molybdate, and 10% glycerol (v/v)]. Supernatants were collected after centrifugation at 105,000 × *g* for 60 min at 4°C and used for steroid receptor and cathepsin D analysis. ER and PR assays were performed, as recommended by the manufacturer (Abbott Laboratories, Chicago, IL), by enzyme immunoassay using monoclonal rat antibodies to MCF-7 human breast cancer (33). The total amount of cathepsin D was assayed by a solid-phase, immunoradiometric method (ELSA-Cath D kit; CIS International, Gif-sur-Yvette, France). The first monoclonal antibody (D7E3) was coated on the ELSA solid phase; the second monoclonal antibody (M1G8), which was radiolabeled with ^{125}I, was used as a tracer. Protein concentration was determined using the Coomassie brilliant blue method (Ref. 34; Bio-Rad, Richmond, CA).

ODC activity was assayed as follows: a portion of the tumor was homogenized in 20 volumes of ice-cold 0.25 M sucrose, 25 mM Tris-ClH buffer (pH 7.2), 2 mM DTT, 0.1 mM EDTA, and 0.1

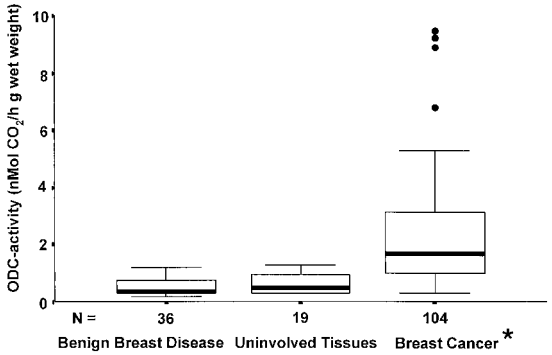

Fig. 1 Box plot of ODC activity in breast cancer, benign breast diseases, and uninvolved breast tissue. In this representation, the central box covers the middle 50% of the data values, between the upper and lower quartiles. The *bars* extend out to extremes, whereas the *central line* is at the median. Values that are 1.5 times the interquartile range beyond the central box are plotted as individual points. *N*, number of patients. *, $P < 0.001$ *versus* the other groups.

Table 2 Polyamine concentrations in breast cancer and in benign breast disease[a]

Variable	Mean ± SE	Median	Range
PUT			
Benign disease	42.2 ± 3.9[b]	41.6	5.6–101.5
Cancer	101.3 ± 10.2	82.5	26.5–204.6
SPD			
Benign disease	90.4 ± 10.1[b]	76.8	56.4–156.3
Cancer	222.0 ± 13.5	193.7	95.6–759.3
SPN			
Benign disease	128.0 ± 22.2[b]	115.2	65.4–210.5
Cancer	310.5 ± 18.6	264.3	95.3–790.6
Total polyamines[c]			
Benign disease	295.8 ± 27.4[b]	195.4	104.6–426.8
Cancer	704.3 ± 38.3	610.3	356.4–1845.0

[a] Number of patients: 36 with benign breast diseases (including 21 fibroadenomas and 15 fibrocystic diseases) and 104 with breast cancer.
[b] Statistical significance, Student's *t* test, $P < 0.001$.
[c] Polyamines are expressed as nmol/g wet weight.

mM pyridoxal phosphate. The extract was centrifuged for 1 h at 105,000 × *g* and the supernatant fraction was used as the enzyme source. ODC activity was determined according to Russell and Snyder (35), by measuring the release of $^{14}CO_2$ from 38 μM 1-^{14}C-L-Ornithine (specific activity, 56 Ci/mol). $^{14}CO_2$-release was totally inhibited by 0.1 mM α-DFMO. In large tumors, different homogenates from small portions from the same tumor gave ODC values with a variation coefficient <10%.

For polyamine analysis, a portion of the tumor was homogenized in 10 volumes of 0.4 M perchloric acid, and the supernatant obtained after centrifugation for 5 min at 8000 × *g* was used for polyamine determination, following the dansylation method of Seiler (36). Dansylated polyamines were separated by high-performance liquid chromatography, using a Lichrosorb 10-RP-18 column (4.6 × 250 mm) and acetonitrile:water mixtures (running from 70:30 to 96:4 ratios during 25 min of analysis) as mobile phase. 1,6-Hexanediamine was used as internal standard. Detection of the derivatives was achieved using a fluorescence detector with a 340-nm excitation filter and a 435-nm emission filter.

Portions weighing 400–500 mg were big enough to carry out the analytical assays described above. Tumors graded as T_1 used in this study (see Table 1) had sizes between 14 mm and 20 mm.

DNA content and cell cycle analysis of the tumor cells were estimated by flow cytometry. Tumor cell nuclei were obtained from formaldehyde solution-fixed, paraffin-embedded tissue according to the method of Hedley *et al.* (37). The nuclei suspensions were stained with a solution containing propidium iodide (50 μg/ml), RNase (100 units/ml), and NP40 (0.05%) for at least 20 min at 4°C. Human normal lymphocytes were used as an external standard. DNA content was measured with a FACScan flow cytometer (Becton Dickinson, San Jose, CA), and a total of 30,000 nuclei was acquired for each sample. Tumors were scored as DNA-diploid when the DNA index was between 0.95 and 1.05 and as DNA-aneuploid when the index fell outside this range or when two distinct peaks could be discerned. The percentage of nuclei corresponding to the SPF was calculated planimetrically.

Statistical Analysis. Statistical differences within the population were determined by Student's *t* test and Mann-Whitney *U* test. Differences in the distribution of characteristics among patient subgroups were analyzed using the Pearson's χ^2 test. Continuous variables were transformed into binary variables, the cutoff values were determined using the graphic method, several variable values were plotted against the *P* of the differences of percentage of relapse and death between variable+ and variable−. For each parameter, actuarial disease-free survival and overall survival were calculated according to the method of Kaplan and Meier and compared using the log-rank test.

The relative importance of the prognostic factors was assessed in a multivariate analysis by the Cox proportional hazards regression model in a forward stepwise procedure. All computations were carried out using the SPSS statistical software.

RESULTS

ODC activity in human breast cancer tissues ranged from 0.51–10.31 nmol CO_2/h g, with a mean value of 2.42 ± 0.22 nmol CO_2/h g (mean ± SE). This activity was significantly higher than that found in benign breast disease, such as fibroadenoma and fibrocystic disease (0.62 ± 0.15 nmol CO_2/h g), or in benign breast tissue adjacent to the primary carcinoma (0.52 ± 0.16 nmol CO_2/h g; Fig. 1). The analysis of ODC activity in breast tissues from 19 patients showed that there is no correlation between enzyme activity in cancer tissue and that of uninvolved breast tissue (Pearson, $r = 0.14$; $P = 0.29$). The levels of PUT, SPD and SPN, and total polyamines in breast carcinoma were significantly higher than those found in benign breast diseases (Table 2). Positive correlations were found between ODC activity and the concentrations of PUT, SPD, and SPN in breast cancer tissues (Pearson: PUT, $r = 0.39$; SPD, $r = 0.49$; SPN, $r = 0.43$; $P < 0.001$). A comparison of tumor ODC activity between premenopausal and postmenopausal patients showed that ODC activity in the premenopausal group (3.27 ± 0.61 nmol CO_2/h g) was significantly higher ($P < 0.02$) than that found in postmenopausal patients (2.12 ± 0.15 nmol CO_2/h g). No significant differences in polyamine levels between the two groups were observed. Polyamine concentrations in blood

Table 3 χ^2 analysis of ODC, polyamines, and other clinical, histological, and biochemical factors in breast cancer

	Tumor size	Node status	HG	ER	PR	PLBI	DNA ploidy	SPF	Cathepsin D	ODC activity	Total polyamines
Tumor size											
Node status	0.009										
HG[a]	NS[b]	0.004									
ER[c]	0.02	NS	0.0001								
PR[d]	NS	NS	0.005	0.001							
PLBI	0.001	0.001	0.002	NS	NS						
DNA-ploidy	NS	NS	0.005	NS	0.01	NS					
SPF	NS	NS	0.01	NS	NS	0.02	NS				
Cathepsin D	NS	NS	NS	NS	NS	NS	NS	0.03			
ODC activity	NS	NS	NS	NS	NS	0.001	0.003	0.0001	0.009		
Total polyamines	NS	NS	NS	NS	NS	0.01	0.0002	0.001	0.013	0.0005	

[a] By the method of Scarff, Bloom, and Richardson.
[b] NS, not significant ($P > 0.05$).
[c] Positive if ≥10 fmol/mg protein.
[d] Positive if ≥10 fmol/mg protein.

Table 4 Univariate analysis of ODC activity and polyamines in breast cancer

		Disease-free survival (%)		Overall survival (%)	
Variable	Patients	5-yr rate (SD)	*P*	5-yr rate (SD)	*P*
ODC activity			0.001		0.0001
<2.5 nmol CO_2/h g	67	67.6 (6.3)		78.1 (5.2)	
≥2.5 nmol CO_2/h g	37	28.1 (8.6)		55.8 (8.3)	
PUT			0.03		0.068
<105 nmol/g	62	65.4 (6.9)		76.1 (5.3)	
≥105 nmol/g	42	33.6 (8.7)		57.9 (7.9)	
SPD			0.48		0.87
<230 nmol/g	65	58.2 (6.6)		74.3 (5.9)	
≥230 nmol/g	39	46.1 (9.5)		69.5 (7.8)	
SPN			0.43		0.79
<340 nmol/g	67	60.3 (6.3)		71.2 (5.5)	
≥340 nmol/g	37	42.5 (9.4)		73.1 (7.5)	
Total polyamines			0.25		0.69
<705 nmol/g	65	61.6 (6.5)		73.6 (5.5)	
≥705 nmol/g	39	41.1 (8.9)		70.9 (7.4)	

from patients with breast carcinoma were not significantly different with respect to those found in control women (results not shown).

Breast carcinoma samples (66.3%) presented ODC values >1.2 nmol CO_2/h g, the cutoff value obtained from the 95 percentile of the ODC values observed in benign breast diseases. This value rose to 92.3% for total polyamine content (cutoff = 405 nmol/g wet weight). These values were significantly higher than that found for cathepsin D (43.7%, cutoff = 54 pmol/mg protein), a protease used as an indicator of malignancy (38, 39).

Table 3 shows the correlations of ODC and total polyamines with several other biochemical and histopathological parameters analyzed in breast cancer tissues. ODC activity correlated well with total polyamines concentration, DNA ploidy, percentage of cells in S phase, PLBI, and cathepsin D. No correlation was found among ODC activity and tumor size, node status, or steroid (estrogen and progesterone) receptor status. Although a poor correlation was found between ODC activity and HG by χ^2 analysis ($P = 0.09$), ODC activity in tumors of grade III (3.15 ± 0.62 nmol/hg, $n = 23$) was significantly higher ($P < 0.001$) than in tumors of grade I (1.63 ± 0.23 nmol/h g, $n = 13$). The relative large number of samples with grade II and intermediate ODC level (2.27 ± 0.34 nmol/h g, $n = 68$) may account for this apparent discrepancy.

Total polyamine concentration increased with SPF (>10%), cathepsin D activity (≥60 pmol/mg protein), PLBI, and ploidy (tetraploid tumors having the highest amount of polyamines, 994 ± 20 nmol/g wet tissue). No correlation between total polyamines and tumor size, node status, HG, or hormone receptor status was observed (Table 3). However, as found for ODC activity, SPN concentration in poorly differentiated tumors (HG III) was significantly higher ($P < 0.001$) than that found in the highly differentiated grade I tumors (370 ± 21 nmol/g and 247 ± 17 nmol/g, respectively).

Univariable analysis revealed that both recurrence and survival correlated well with ODC activity (Table 4). Patients with higher ODC values presented a higher percentage of recurrence and death. Polyamine analysis showed that only increased PUT was associated with an increased risk of recurrence and death.

Multivariate analysis of all parameters studied, including adjuvant therapy, indicated that ODC activity, the axillary status, and DNA ploidy were significant prognostic factors for

Table 5 Prognostic factors in breast cancer patients

Median follow-up was 63.4 months. The multivariate analysis was performed with Cox's model, with the variables entered stepwise. Hazard ratio is presented only for the retained variables.

	Disease-free Survival			Overall Survival		
	P	Hazard ratio (95% CI[a])	χ^2	*P*	Hazard ratio (95% CI)	χ^2
Overall population						
Axillary status	0.0001	2.07 (1.45–2.95)	19.68	0.0007	2.20 (1.36–3.54)	13.19
DNA ploidy	0.0010	1.86 (1.28–2.68)	13.89	0.0078	1.73 (1.15–2.61)	8.36
ODC activity	0.0031	1.66 (1.18–2.34)	5.40	0.051	1.23 (1.07–1.98)	2.37
ER	0.072	1.06 (0.96–1.93)	1.72	0.0012	1.92 (1.31–2.79)	11.51
Node-negative patients						
ODC activity	0.022	2.04 (1.10–3.78)	5.64	0.93	1.04 (0.83–1.65)	2.71

[a] CI, confidence intervals.

disease recurrence. For overall survival, the prognostic value of ODC was inferior to that given by the number of axillary nodes, DNA ploidy, or ER status (Table 5).

Interestingly, ODC activity was the only significant prognostic factor of disease-free survival in node-negative patients. Total polyamine concentration was a significant prognostic factor for death in node-positive patients (hazard ratio, 1.09; $P < 0.039$).

Fig. 2, *A* and *B*, shows the relation of tumor ODC activity with overall survival and with relapse-free survival. It can be seen that patients with higher values of ODC activity present significantly higher taxes of recurrence and death than those having lower ODC values.

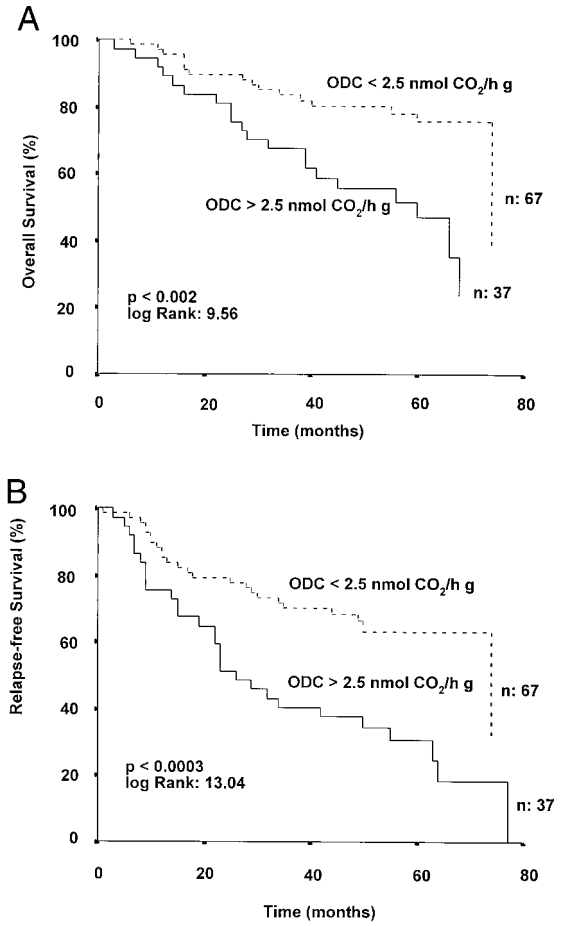

Fig. 2 Survival curves for overall survival (*A*) and relapse-free survival (*B*) in ODC activity subgroups of primary breast carcinoma patients.

DISCUSSION

The present study demonstrates that ODC activity and polyamine levels in human breast cancer tissues are considerably higher than those found in benign mammary disease tissues or in normal breast tissue surrounding primary breast carcinomas. Although in normal breast tissues the lower levels in ODC activity and polyamines found may be related to the low cellularity of these samples, specimens from benign mammary pathology with a cellular content close to those breast carcinomas still presented significantly lower values of ODC and polyamines. Our results are generally in agreement with other studies in which either ODC activity (29, 30) or polyamines (16, 17) in human breast cancer have been measured. Our study, apart from including a larger number of patients, also shows that there is a positive correlation between tumor ODC activity and the individual and total polyamine concentrations in primary human breast cancer specimens. The fact that blood polyamines are not raised in patients with breast cancer before surgery, despite the high values found in the tumor samples, indicates that in this carcinoma blood polyamines cannot be considered as a marker of malignancy. The fraction of patients with elevated ODC activity and polyamines in breast carcinoma was higher than the one found for cathepsin D, a biochemical indicator of malignancy (38, 39). This suggests that the analysis of both ODC and polyamine content in biopsy specimens may have interest in the diagnosis of malignancy and prognosis. The elevated values of ODC and polyamines in human breast tumors found in this study are consistent with the findings that the overexpression of ODC leads to the transformation of cultured cells (20, 21) and enhancement of tumorigenicity (23, 24, 40).

The results also show that SPN is the most abundant polyamine in breast carcinomas and that the SPD:SPN ratio, considered as a proliferative index in some tumors, has a similar value in normal breast tissues than in breast cancer tissues. This result, together with the high variability in the SPD:SPN ratio observed in different models of animal mammary tumors (11, 13), suggest that the SPD:SPN ratio is not a reliable index of cell proliferation in

breast tissue. The finding showing a lack of correlation between ODC activity in primary human breast cancer specimens compared with surrounding normal breast tissue is in agreement with the idea that the presence of a carcinoma in the breast does not modify polyamine levels in the surrounding uninvolved tissue (16). Similar results have been described for SPD/SPN *N*-acetyltransferase (41). These findings are in contrast with reported data on human gastrointestinal types of cancer in which ODC activity measured in the normal-appearing mucosa obtained from cancer-bearing patients was higher than that found in mucosa from patients without cancer (42, 43).

The importance of estrogens in the progression of human breast cancer is very well known (44). The estrogenic regulation of cell growth is interlinked with the polyamine pathway (8, 45, 46). Studies in animal models have shown that the hormonal activation of breast cancer growth is completely abolished by treatment with α-difluoromethylornithine, which suggests that polyamines are involved in the hormonal stimulation of neoplastic cell proliferation (11). Despite these findings, the influence of steroid hormones and hormone receptors on polyamine metabolism in human breast cancer *in vivo* is unclear. Our results indicate that the hormonal status may affect tumor ODC activity because the enzymatic activity was higher in premenopausal than in postmenopausal patients. However, the lack of correlation between ODC activity and the tumor content of ERs and PRs found in our study is in agreement with that reported by others (29). In this regard, no significant correlation between hormone receptors and polyamine levels in mammary tumors developed in rats has been reported either (10). All these results indicate that the plausible relationship between hormones and polyamines in human breast cancer seems to be more complex than the one observed in cultured cells.

Neither ODC activity nor total polyamine content correlated with tumor size or nodal status, two parameters related with the extent of the disease. This is in agreement with other reported results found in human breast and liver carcinomas (29, 47), although positive correlation between tumor size and polyamine content has been found in pancreatic cancer (48).

In our study, the HG correlated well with ODC activity, which is in agreement with other studies that have shown that ODC activity is higher in poorly differentiated tumors (29, 47). These results would support the notion that in tumors with higher cellularity and nuclear anaplasia (HG III) the fraction of cells with rapid cytokinetics may be important. This also agrees with our finding that tumors with a higher ratio of S phase cells presented higher ODC activity and polyamine content. Cell cycle experiments have clearly shown an increase in ODC activity at the beginning of S phase (49), whereas in quiescent human cells the expression of the *ODC* gene was almost undetectable (50). These facts and the higher polyamine levels found in aneuploid tumors would suggest that elevated ODC activity and high polyamine content in breast cancer tissues could correlate with higher aggressiveness.

Interestingly, tumors with positive PLBI presented significantly higher values of ODC activity and increased amounts of polyamines. In human hepatocellular carcinoma, ODC activity was also significantly higher in tumors that demonstrated portal invasiveness (47). Although different studies have shown that ODC can be considered as an oncogene playing an important role in malignant cell transformation (20, 21), the function of ODC in tumor metastasis is unclear (24). The recent demonstration that ODC is directly involved in mouse mammary carcinoma cell invasion *in vitro* (51) and the reported antimetastatic effects of DFMO (an irreversible inhibitor of ODC) on pulmonary metastasis induced by injection of B16 melanoma or Lewis lung carcinoma cells *in vivo* (6) would support the notion that ODC may be implicated in the invasiveness of breast cancer cells. Furthermore, it has been proposed that the induction of the angiogenic phenotype in ODC-overexpressing cells may be due to the increased expression and secretion of a new angiogenic-stimulating factor and to a decreased production and release of the antiangiogenic thrombospondins (52).

Our results demonstrate that ODC activity in human breast cancer is a negative independent prognostic factor for both disease-free survival and overall survival. This corroborates and extends the finding of a previous study supporting the prognostic role of ODC in human breast cancer (30). In our analysis, ODC activity was as good a predictor of recurrence as the axillary node status, DNA ploidy or ER status. Interestingly, ODC activity was the only independent prognostic factor of disease-free survival in axillary node-negative patients.

In conclusion, our results on polyamine metabolism in human breast carcinoma corroborate the idea raised by others (53) that the measurement of ODC activity in biopsy specimens from human neoplasms can be very useful in clinical oncology for determining the degree of malignancy and prognosis. These results also support the contention that the polyamine pathway and in special ODC may be an adequate target for adjuvant therapy or chemoprevention in human cancer (54).

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