Alterations in Polyamine Catabolic Enzymes in Human Breast Cancer Tissue

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ABSTRACT

High concentrations of acetyl polyamines have been observed in human breast cancer compared to the equivalent normal tissue, however, no explanation as to the reason for the increases has been proposed. In this study, we show that changes in the enzymes responsible for the breakdown of acetyl polyamines occur in breast cancer tissue. Spermidine/spermine N\(^1\)-acetyltransferase, the first and rate-limiting enzyme in polyamine catabolism, is increased in the tumor tissue whereas polyamine oxidase (PAO) is decreased. The changes in PAO correlate with prognostic factors, and activity decreases as the size and histological grade of tumors increase. The metabolism of polyamines by PAO generates locally high concentrations of hydrogen peroxide, a known inducer of apoptosis; thus, low PAO activity may contribute to the low level of apoptosis seen in tumor cells. Therefore, drugs that induce PAO activity may be a novel means of attacking tumor cells.

INTRODUCTION

Intracellular concentrations of the naturally occurring polyamines, spermidine and spermine and their diamine precursor, putrescine, have been closely linked with the rate of cell growth and differentiation (1). In general, high concentrations of polyamines are found in rapidly growing cells, and low concentrations are present in quiescent cells. Many types of cancer cells, including breast (2) and colon (3), have also been reported to have a high intracellular polyamine content, although the function of these increased concentrations of polyamines is not clear. Our particular interest has been in breast cancer where increases of 3–4-fold were observed in all three polyamines in the malignant tissue compared with equivalent normal tissue (2).

In addition to the naturally occurring free polyamines, mammalian, and some bacterial, cells can synthesize a number of monocacyl polyamine derivatives (4). In normal cells acetylation of the polyamines is part of the catabolic process to remove polyamines from the cell, and little, if any, acetyl polyamine derivatives are detected (5, 6). In contrast, in some tumor cells, significant quantities of acetyl polyamines have been found. In breast cancer cells the amount of acetyl polyamine detected was high, greater, in fact, than some of the free polyamines (7). Acetyl polyamines are synthesized by the action of the enzyme SSAT\(^3\), which forms part of the retroconversion pathway converting the higher polyamines spermine and spermidine back to putrescine (8). These acetyl derivatives are then the preferred substrate for the second catabolic enzyme, PAO. Acetyl polyamines are, however, also substrates for the outward transport of the polyamines (9), which removes excess polyamine from the cell in times of growth limitation (10). Thus, in normal cells a combination of SSAT, PAO, and polyamine export results in very low or nondetectable levels of acetyl polyamines. Therefore, in breast cancer cells where high concentrations of acetyl derivatives are found, one or more of these processes must be disrupted or deregulated.

The aim of this study was to compare SSAT and PAO enzyme activities in human breast cancer tissue with equivalent nonmalignant breast tissue in an attempt to explain the high levels of acetyl polyamine in this malignant tissue.

MATERIALS AND METHODS

Patients. Patients were admitted to the Breast Unit, Aberdeen Royal Infirmary, for the staging and definitive treatment of carcinoma of the breast. Investigations carried out in all patients to determine the presence of detectable metastatic disease were hemoglobin and full blood count, urea and electrolytes, liver function tests, chest radiograph, and isotope bone scan. The size of the breast tumor was measured clinically using the TNM system.

Immediately after removal of the tumor from the patient, a tumor sample measuring ~5 mm in diameter was removed from the tumor mass, immediately cooled to 0°C, and then stored at ~80°C until analyses were undertaken. In addition, a piece of normal breast tissue (1 cm in diameter) was removed as far away as possible from the quadrant of breast containing the tumor. This was treated in the same fashion as the tumor sample.

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3 The abbreviations used are: SSAT, spermidine/spermine N\(^1\)-acetyltransferase; PAO, polyamine oxidase; TNM, tumor, node, metastasis.
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started by the addition of 2 mM peroxidase. The reaction was carried out in low light and was contained 10 mM Tris-HCl, 2.5 mM DTT, and 1 mM EDTA for washing twice in ice-cold PBS and incubated in hypotonic buffer containing 0.1% Triton X-100. PAO activity was measured in the crude homogenate for SSAT activity due to insufficient nonmalignant tissue being available. Results are mean ± SEM with the n value as shown in the table.

### Enzyme Assays
SSAT and PAO activity was measured in tumor samples and in histologically graded normal tissue.

**SSAT Activity**. Tumor tissue was finely chopped and washed twice in ice-cold PBS and incubated in hypotonic buffer containing 10 mM Tris-HCl, 2.5 mM DTT, and 1 mM EDTA for 15 min at 4°C. Tissue was then homogenized using an Ultra Turrax and a cytosolic extract prepared by centrifugation at 100,000 g for 1 h. The cytosolic supernatant was then assayed for SSAT activity, as described previously (11). Protein content of the tissue homogenate was determined by the method of Lowry et al. (12). Each sample was assayed in duplicate, and the results are expressed as pmol of N\textsuperscript{3}-acetylsperrmidine formed/min/mg protein.

**PAO Activity**. Tumor tissue was roughly chopped and washed twice in ice-cold PBS. It was homogenized in 10 mM Tris-HCl buffer containing 0.1% Triton X-100 using an Ultra Turrax. PAO activity was measured in the crude homogenate fraction and in the supernatant, and the pellet of a subcellular extract prepared by centrifugation at 100,000 g for 80 min. The assay contained 2 mM aminoguanidine, 2 mM pargyline, 10 mM sodium borate buffer (pH 9.0), and 90 IU of horseradish peroxidase. The reaction was carried out in low light and was started by the addition of 2 mM N\textsuperscript{3}-acetylsperrmidine and 9.2 mM homovanillic acid. After 30 min, the reaction was stopped with 20 mM NaOH and the fluorescence was measured at excitation wavelength 323 nm and emission wavelength 426 nm. Protein content of the homogenate was measured as described previously (12). Each sample was assayed in triplicate, and the results are expressed as pmol/min/mg protein.

Statistical differences were analyzed by Wilcoxon matched pairs signed rank test or by Mann Whitney U test.

### RESULTS
A total of 22 patients with a mean age of 71 ± 3 years (SEM) were included in the study. The clinical tumor size, histological presence of lymph node involvement with tumor, and presence of detectable metastatic disease are shown in Table 1 for all patients. The tumor type for all patients is also shown, and where tumors were invasive ductal carcinomas these were graded as grade 1, 2, or 3 using standard criteria.

SSAT activity was significantly higher (P < 0.02) in tumor tissue compared with the equivalent normal tissue (Table 2). Changes in the activity between normal and tumor tissue for individual patient samples are shown in Fig. 1a. PAO activity, on the other hand, was significantly lower (P < 0.001) in tumor tissue (Table 2). Again, individual changes between normal and tumor activity are shown in Fig. 1b. Both SSAT and PAO activities were then related to prognostic factors.

SSAT activity did not correlate with age in either normal or tumor tissue (Fig. 2a). In tumor tissue, however, PAO activity showed a significant negative correlation with age using the Mann Whitney U test (P < 0.001; Fig. 2b), however, no relationship existed between PAO activity in nonmalignant tissue and age (Fig. 2b).

For all tumor sizes PAO activity was significantly lower in the tumor samples than in the normal tissue (Fig. 3a). Similarly for SSAT activity, the activity in the tumor tissue was higher than in the nonmalignant tissue (Fig. 3b). In tumor tissue the trend was for the larger the tumor size the lower the PAO activity (Fig. 3a) and the higher the SSAT activity (Fig. 3b). As a result of the large interindividual variation only the comparison of activity in nonmalignant versus tumor SSAT activity for T2 tumors was statistically significant (P < 0.05).

When comparing PAO activity with histological grade there was an inverse relationship: the higher the grade, the lower the PAO activity (Fig. 4a). Similarly for SSAT activity, the activity in the tumor tissue was higher than in the nonmalignant tissue (Fig. 4b). In tumor tissue the trend was for the larger the tumor size the lower the SSAT activity (Fig. 3a) and the higher the SSAT activity (Fig. 3b). As a result of the large interindividual variation only the comparison of activity in normal versus tumor SSAT activity for T2 tumors was statistically significant (P < 0.05).

### DISCUSSION
The high concentrations of acetylpolyamine derivatives found in tumor tissue (7) may be derived in four ways: by increased synthesis, by decreased degradation, by increased uptake of polyamines, or by decreased export of acetylpoly-
amines. The first two possibilities can be tested in man by measuring the activity of the polyamine catabolic enzymes PAO and SSAT in tumor tissue and comparing the activities with those found in equivalent nonmalignant tissue. Uptake and export in man is more difficult to quantify and most studies in this area have been carried out using human cancer cell cultures (10). All four mechanisms could contribute to the overall increased acetylpolyamine content of breast tumors.

In this study, both PAO and SSAT activities were altered by the presence of the tumor burden (Table 2). In terms of the relative contribution of each reaction, the greater effect seems to be on PAO activity where the decreases are larger. It has been proposed that the hydrogen peroxide produced during the oxidation of polyamines by PAO may contribute to the level of apoptosis (13, 14) that occurs in human cells. Thus, it is tempting to speculate that the significant decreases in PAO activity seen in breast cancer tissue may contribute to tumor growth through a decreased rate of endogenous apoptosis resulting from decreases in the local concentrations of H₂O₂. Recent work from our laboratory has shown that an early response in human tumor cells to etoposide-induced apoptosis is an increase in PAO activity (14), thus supporting a link between PAO and apoptosis. On the other hand, increases in SSAT activity have been linked to cytotoxicity in some cell types (15, 16). Recent data from our laboratory suggest that increases in SSAT are more likely to be a protective mechanism in cells with SSAT producing acetyl derivatives for export or for recycling when polyamines are present in excess of cellular requirements (14). When combined, SSAT and PAO can produce an efficient system to generate locally high concentrations of hydrogen peroxide that could effectively be a death signaling pathway. In these breast cancer cells this normal death-generating pathway is blocked by the decrease in activity of PAO and, thus, acetylpolyamines accumulate within the tissue. The acetylpolyamines, unlike the free polyamines (17), are not significantly toxic to tumor cells⁴ and may, thus, remain within the tumor cells without causing any negative effects.

Fig. 1 Changes in individual SSAT (a) and PAO (b) activity in nonmalignant versus tumor samples. Samples (nonmalignant; tumor) were collected as described and stored at −80°C until assayed for SSAT or PAO activity, as described in “Materials and Methods.”

⁴Wallace et al., unpublished results.
Both enzyme activities correlate with prognostic factors: PAO in a negative manner and SSAT in a positive manner supporting the idea that polyamine catabolism, particularly oxidation, is linked to tumor growth potential. It is interesting that a negative correlation was observed between PAO activity and age (Fig. 1a). If oxidation of polyamines does generate locally high concentrations of H2O2, then low PAO activity will correlate with low H2O2 production and lower apoptosis. Low rates of apoptosis are associated with cancer progression, and cancer is, in general, a disease of older age; thus, it is tempting to speculate that the decrease in PAO activity in normal tissue may increase the susceptibility of this tissue to unrepaired mutational events.

In this study, we attempted to measure PAO activity in serum samples from patients, with the aim of this being a useful marker of disease progression. However, in agreement with others (18), the PAO activity in serum was very low and, therefore, not a reliable index of tumor burden.

In summary, PAO activity is decreased in breast cancer tissue, and this decrease correlates positively with aggressiveness of the tumor. SSAT activity is increased in breast tumor tissue and shows a tendency to increase further with factors indicative of a poor prognosis.

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