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## Acid Phosphatase Activity in Human Breast Tumors

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## Abstract

We examined lysosomal participation in the degradation of tumor cells from human breast biopsies, utilizing the histochemical activity and localization of acid phosphatase (AP). Enzyme activities in benign and malignant lesions were compared. AP was faintly detected in normal mammary epithelia and was marked in malignant cells. The histochemical patterns of AP distribution in the breast tissues showed differences between normal and neoplastic cells. AP staining was more intense in ductal carcinoma *in situ* (DCIS) associated with invasive ductal carcinoma (IDC) cases compared to when IDC was present alone. These results suggest that non-invasive breast cancers, such as DCIS, may be subjected to more lysosomal cellular lysis than is observed in invasive breast cancers, like IDC.

## Keywords

acid phosphatase, lysosomes, breast cancer, ductal carcinoma in situ, invasive ductal carcinoma

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## Introduction

It has been shown that lysosomes, acting presumably via their acid hydrolases, are involved in a variety of cytoplasmic degradative changes during physiological processes [1, 2] and regression of mammary tumors [3, 4]. Acid phosphatase (AP) has also been used to monitor cell death and cell lysis [5,6,7,8]. Activities of lysosomal hydrolases were demonstrated to be more marked in cancer cells than in homologous normal tissue [9, 10]. Ductal carcinoma *in situ* (DCIS), also known as intraductal carcinoma, is characterized by proliferation of presumably malignant epithelial cells within the mammary ductal-lobular system, without light microscopic evidence of invasion into surrounding stroma [11]. An increasing number of women have been diagnosed with DCIS as more sophisticated methods for detecting breast cancer are being used. Autopsies performed on women who died from all kinds of causes showed that 6-16 % of them had DCIS [12, 13]. Over 14 % of breast cancers diagnosed in the United States annually are DCIS [14]. Some DCIS lesions, if left untreated, may progress to

invasive breast carcinoma [15]. Invasive ductal carcinoma (IDC) often metastasizes to the axillary lymph nodes and is associated with a relatively poor prognosis compared with other types of breast cancer, such as medullary or tubular carcinoma [16]. Other common sites of metastases for IDC include bone and intraparenchymal sites within the lung, liver and brain [16].

In this report, AP, the marker enzyme for lysosomes [17], was used to visualize these organelles in normal and neoplastic breast tissues. We detected higher AP activity and histochemistry in DCIS associated with IDC compared to IDC alone.

## Materials & methods

### Tissue Collection & Processing

Normal and malignant breast tissues used in this study were obtained from the Cooperative Human Tissue Network (Philadelphia, PA). Two cases of IDC associated with DCIS and one case of IDC were studied. The patients were 58, 67, and 73 years old at the time of the biopsies. Normal breast tissue from each patient was also examined and considered as controls. Tissues were fixed overnight in 4 % paraformaldehyde at 4°C, placed in Tissue Tek OCT (Miles, Elkhart, IN), frozen in liquid N<sub>2</sub>, cut as 5 µm sections onto poly-L-lysine coated slides (Sigma, St. Louis, MO), and stored at -20°C until they were stained.

### Lysosomal Localization

Lysosomes were localized from slides of frozen sections using a histochemical assay for AP (Sigma) as previously described [18]. Briefly, prior to fixing the slides, 0.6 mL of sodium nitrite solution were added to 0.6 mL of fast garnet GBC solution, mixed by inversion; allowed to stand for 2-4 min; and added to 23 mL of dH<sub>2</sub>O. Three mL of acetate solution and 3 mL of naphthol AS-BI phosphoric acid solution (provides naphthol AS-BI phosphate, the substrate) were added to the previous solution. Slides were fixed in citrate-acetone-formaldehyde solution at room temperature, in Coplin jars, for 30 sec and rinsed with dH<sub>2</sub>O for 1 min. Slides were then incubated for 1 h at 37°C in naphthol AS-BI phosphate and fast garnet stain in acetate buffer. Slides were rinsed with tap H<sub>2</sub>O for 2 min, dried for 15 min, counterstained with methylene blue for 1 min, rinsed with dH<sub>2</sub>O, and mounted in CrystalMount (Biomedica Corp., Foster City, CA). The presence of acid phosphatase was indicated by distinct red-violet focal precipitates, which were resolved by light microscopy.

## Results and discussion

Normal myoepithelial cells showed a moderate amount of lysosomes (Figs. 1,2,3, panels a and b). The increase in lysosomes was apparent in the tumor cells. The acid phosphatase preparations revealed conglomerates (dark granules) in tumor cells from patients with DCIS associated with IDC (Figs. 2 and 3, panels c and d). However, less AP activity was detected in the case of IDC alone (Fig. 1, panels c and d). The tumor cells displayed histochemical, degenerative changes (Figs. 2 and 3, panels c and d). The concentration of AP particles, which characterize lysosomes, was increased in breast tumor cells. Cords of tumor cells were strongly positive for AP, while the stromal tissue was unstained (Figs. 2 and 3, panels c and d). The positive granules are much larger in the breast tumor cells than those in the normal breast cells (Figs. 1,2,3).

**Figure 1** Infiltrating Ductal Carcinoma (IDC). a, b) Faint histochemical activity (dark granules) is seen in the epithelial cells from normal breast tissue and is negative in the connective tissue.. c) The florid complex and irregularly shaped epithelial bridges resulted in superimposed micropapillary features. d) Enhanced enzyme activity is evident in the epithelial elements. X 200, a and c; X 400, b and d.

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**Figure 2** IDC Associated with Ductal Carcinoma in situ (DCIS). a, b) Very few AP positive granules (dark granules) are seen in the normal breast. Stromal fibroblasts contain no enzyme activity. c, d) Cords of tumor cells are strongly positive while the stromal tissue is unstained (clear areas). X 200, a and c; X 400, b and d.

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**Figure 3** IDC Associated with DCIS. a, b) Normal breast myoepithelia has is sparsely stained for AP. c) Strong enzyme activity is evident throughout the cytoplasm of the tumor cells. The AP positive granules (dark areas) are much larger than those in the control tissue (a, b). The majority of the tumor cells are AP positive, suggesting that they are undergoing cell death. d) Intense AP activity in the cords of tumor cells. Note that AP staining is absent in the stroma. X 200, a and c; X 400, b and d.

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Histochemical reactions for acid phosphatase were demonstrated in all cases of breast cancer that we examined. The reaction was most constant in the epithelial cells and negligible in connective tissues. This observation is in agreement with previous reports [19, 20]. It seems that increased acid phosphatase activity is a constant feature in neoplastic transformation [21], and this was noted in all the cases of breast carcinomas we studied. Acid phosphatase activity has been used to indicate that lysosomes participate in the execution of cell death in a variety of tissues [22, 23] and in the regression of mammary carcinomas [24, 25]. Apoptosis could represent a barrier to the progression of invasive cancer [26, 27]. Our observations support this hypothesis in that we detected more AP activity in DCIS relative to IDC. Moreover, a recent study found that apoptosis comprises only 11 cells per mm<sup>2</sup> of tissue in 288 cases of invasive breast cancer [28]. Acid phosphatase has been used as a marker of metastatic bone disease and response to treatment in breast cancer patients [29]. In this light, our findings suggest that the level of AP activity in breast tumors may be correlated to the amount of apoptosis that is going on in the cells.

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