Detection of the Plasmin System in Human Mammary Pathology Using Immunofluorescence

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ABSTRACT

The presence and localization of the plasmin system components urokinase (UPA), tissue type plasminogen activator (TPA), plasminogen (PG), a neogenin expressed by the plasmin α2-antiplasmin complex, and plasmin inhibitors α2-antiplasmin (AP) and α2-macroglobulin (MG) have been tested by immunofluorescence on sections of 11 benign and 40 malignant lesions of the breast in an attempt to apply a morphological approach to the problem of tumor invasion in vivo.

In benign lesions, TPA was seen in secretions of mammary glands and MG was seen in edematous zones. In one involuting lactating adenoma, UPA, TPA, PG, PAP, and AP were associated with glandular cells. UPA was detected in 11 carcinomas, TPA in 22, PG in 31, PAP in 12, AP in 23, and MG in all 40.

All these components were essentially present in invasive territories, with a cellular labeling for UPA and TPA and a fluorescent staining frequently at the periphery of tumor foci for PG and PAP. AP was more closely associated with cancer cells than MG, which was present in the stroma. Intraductal proliferations were rarely positive and there was no correlation between the localization of PG and the distribution of a basement membrane glycoprotein laminin. These data argue strongly for the involvement of the plasmin system in the infiltrating process of the stroma. This system seems to play a limited role in the breakdown of basement membrane in breast carcinomas in vivo.

INTRODUCTION

Invasive tumor cells have the ability to migrate through BM and interstitial connective tissues. Numerous proteinases have been implicated in the local and metastatic spread of cancer including collagensases with special emphasis on type IV collagenase (1), cathepsin B-like enzymes (2, 3), PA leading to the production of active plasmin (4), and others.

Christman (5) demonstrated two types of PA, UPA and TPA. Of these two main types, UPA has been more frequently associated with neoplasia in the literature. Plasmin produced through PA can cleave fibronectin and LM and can also activate a type IV collagenase secreted by tumor cells in a latent form (6). However, the action of plasmin is limited by the inhibitors AP, which is specific and fast-acting (7), and MG, which is nonspecific and also inhibits collagensases and cathepsin B (8, 9).

Most studies on the plasmin system in tumors have been performed either from extracts of tissue or on cell cultures in vitro. Both approaches have drawbacks. Tumors contain many cell types associated with malignant cells, such as inflammatory cells and also adjacent normal tissue, and any assay using tumor homogenates measures the PA activity or the proteinase-inhibitory activity of the whole cell population. Cultures of tumor cells eliminate the contribution of the “contaminating” cells but cells are placed in an artificial environment which may alter their behavior.

These enzymatic and biochemical studies on PA in breast pathology have given various results. Some authors (3, 10) have failed to detect any correlation between neoplasia and PA activity. By contrast, more recent papers have disclosed a UPA activity higher in malignant tumors than in benign lesions (11–13).

The aim of our work was to study the presence and localization of UPA, TPA, PG, PAP, AP, and MG in tumors of the human mammary gland by an immunofluorescence technique. Immunohistochemistry does not indicate enzyme activity, which is dependent on inhibition or substrate limitations and local conditions as well as the presence of the enzyme. Images obtained by immunofluorescence are snapshots of a dynamic enzyme reaction. Enzymes may have a short time of action or be quickly neutralized by inhibitors and so not be detectable. Nevertheless, this morphological approach of the distribution of proteinases, combined in some cases with the concomitant detection of the BM component LM, which is known to interact with PG and TPA (14), may provide a better comprehension of the invasive process.

MATERIALS AND METHODS

Tumors and Tissues. All tissue specimens were obtained as fresh surgical samples. The distribution of UPA, TPA, PG, PAP, AP, MG, fibrinogen, and LM was studied in 1 involuting lactating adenoma, 5 fibroadenomas, 4 samples of fibrocystic diseases without atypical hyperplasia, 1 infiltrating epitheliosis, and 40 carcinomas rated according to the WHO classification (30 ductal invasive carcinomas, 5 lobular carcinomas, 2 ductal invasive carcinomas with intraductal components, 1 medullary carcinoma, 1 colloid mucinous carcinoma and 1 tubular carcinoma).

Antisera. Rabbit antisera reacting specifically against PG, PAP, AP, MG, and fibrinogen were obtained from Behringwerke (Marburg, Federal Republic of Germany). Rabbit anti-TPA antiserum was kindly provided by Dr. D. Collen (University of Leuven, Belgium). Rabbit anti-UPA antiserum was a gift of Dr. P. Burtin (Villejuif, France) and its preparation has been described previously (15). Guinea pig anti-LM purified antibody was a gift of Dr. J. M. Foidart (University of Liège, Belgium) and the preparation of this antibody has been described in a previous paper (16).

Controls. A sample of anti-PG antiserum was passed through an immunosorbent column prepared by coupling 4 mg purified PG obtained from Kabi (Stockholm, Sweden) or specially prepared by the Protein Chemistry Laboratory of the National Blood Bank (C.N.T.S., Les Ulis, France) to activated Sepharose 4B (Pharmacia, Uppsala, Sweden). Before coupling, diisopropylfluorophosphate was added to PG solution to a final concentration of 10−3 M.

Rabbit antiserum against UPA was passed through an immunosorbent column made by coupling 5 mg of purified UPA (a gift from Dr. Harvey, Roswell Park Memorial Institute, Buffalo, NY) to activated Sepharose 4B.

Part of the anti-TPA antiserum was absorbed on an immunosorbent made by coupling TPA, prepared from a human melanoma culture supernatant, to activated Sepharose 4B. This immunosorbent was also donated by Dr. D. Collen.)

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: BM, basement membrane; UPA, urokinase-type plasminogen activator; TPA, tissue-type plasminogen activator; PA, plasminogen activator; PG, plasminogen; PAP, neogenin expressed by plasmin α2-antiplasmin complex; AP, α2-antiplasmin; MG, α2-macroglobulin; LM, laminin; PBS, phosphate-buffered saline.
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Table 1 Detection of the components of the plasmin system in benign proliferations of the breast

<table>
<thead>
<tr>
<th>Lesions</th>
<th>UPA</th>
<th>TPA</th>
<th>PG</th>
<th>PAP</th>
<th>AP</th>
<th>MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactating adenoma (1 case)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fibroadenomas (5 cases)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fibrocystic disease (4 cases)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Infiltrating epitheliosis (1 case)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

* In one case.

Table 2 Localization of the components of the plasmin system in 40 breast carcinomas

<table>
<thead>
<tr>
<th>Localization</th>
<th>UPA</th>
<th>TPA</th>
<th>PG</th>
<th>PAP</th>
<th>AP</th>
<th>MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninvasive cells</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Invasive cells</td>
<td>11</td>
<td>19</td>
<td>31</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Necrotic areas</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Stroma</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>3</td>
<td>19</td>
<td>40</td>
</tr>
<tr>
<td>No. of tumors positive</td>
<td>11</td>
<td>22</td>
<td>31</td>
<td>12</td>
<td>23</td>
<td>40</td>
</tr>
</tbody>
</table>

RESULTS

UPA. In benign proliferations (Table 1), UPA was seen only in the lactating adenoma with a faint staining of cells and glandular lumens. UPA was found in 9 carcinomas (8 ductal and 1 lobular) with a weak cellular staining of some sparse invasive cancer cells. In 2 carcinomas (1 ductal and 1 colloid mucinous), numerous invasive cells were fluorescent (Fig. 1). In all of these 11 tumors, TPA and PG were detected (Table 2).

TPA. In benign proliferations, TPA was present in some vessel walls with staining of the vascular endothelium. It also filled the lumen of numerous normal lobules, especially in the lactating adenoma where glandular cells were stained.

In carcinomas, TPA was also seen frequently in vascular walls. It was detected in malignant cells in 22 carcinomas (18 ductal, 2 lobular, 1 colloid mucinous, and 1 tubular). TPA was present principally in infiltrating clustered or isolated cells with a strong cellular labeling (Fig. 2). In 3 of these tumors, noninvasive cancer cells were stained. Unlike PG or PAP, TPA was distributed not only at the periphery of tumoral foci but also within malignant clumps. TPA was also found as fluorescent deposits in necrotic areas of 6 cancers.

In 2 ductal carcinomas with numerous cancer cells labeled...
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Fig. 3. Ductal carcinoma. PG is found at the periphery of invasive malignant clumps. A, immunofluorescence; B, white light. × 400.

Fig. 4. Lobular carcinoma. Individual cancer cells are labeled with anti-PG antiserum. A, immunofluorescence. B, white light. × 400.

with anti-TPA antiserum, neither PG nor PAP was detected.

PG. In benign proliferations, PG was detected only in the lactating adenoma, at the periphery of involuting lactating glands.

PG has been found in 31 carcinomas (23 ductal, 4 lobular, 1 ductal with intraductal component, 1 tubular, 1 medullary, and 1 colloid mucinous). The number of cells stained varied from one tumor to another. The fluorescence was localized principally in invasive zones, mainly at the periphery of tumoral clusters (Fig. 3), sometimes around individual cells (Fig. 4), and rarely in necrotic areas (2 cases).

In 3 of these carcinomas, PG was seen along the BM, lining intraductal proliferations with an irregular and limited labeling.

In 18 carcinomas, PG was distributed in the stroma with a faint fluorescence, essentially in edematous areas, with a labeling of vessel walls.

PAP. In benign proliferations, only the lactating adenoma expressed labeling with PAP, which showed a faint fluorescence with a pattern of distribution identical to that of PG.

This neoantigen was detected in 12 carcinomas (1 lobular and 11 ductal) which were all positive with the anti-PG antiserum. The fluorescence, which was often weak, was seen around infiltrating malignant cells in all cases and at the periphery of intraductal proliferations in one case. The pattern of distribution was identical to that of PG, but with less extensive staining of cancer cells.

Of those 12 positive carcinomas, TPA was found in 5 cases and UPA in 4 cases.

AP. In benign proliferations, AP was found in the lactating adenoma, 1 fibroadenoma, and 1 sample of fibrocystic disease with a faint irregular labeling of glandular BM. In 23 carcinomas (18 ductal, 3 lobular, 1 medullary, and 1 tubular) either a faint pericellular and stromal labeling around some invasive clustered malignant cells (19 cases) (Fig. 5) or an irregular limited fluorescent staining of the BM zone in intraductal proliferations (4 cases) was seen. The distribution of PAP and AP overlapped.

MG. In all benign proliferations, MG was present in many places in the stroma but without close association with mammary cells.

All carcinomas expressed a more or less intense and extensive fluorescent staining of their stroma. MG was sometimes in close contact with invasive (13 cases) and noninvasive (6 cases) cancer cells (Fig. 6). It was also found in the vascular lumen of some capillaries and in necrotic areas (4 cases).

Fibrinogen. In benign proliferations and carcinomas, fibrinogen was found principally in edematous areas, but its distribution was completely different from that of PG.

Double Staining Experiments. There was no close relationship between the distribution of PG and the localization of LM, especially in noninvasive lesions, where LM expressed a disrupted pattern in BM without any PG detection (Fig. 7). In addition, PG and LM were concomitantly visualized around some well differentiated tumor clumps in some invasive territories.

DISCUSSION

To our knowledge, no immunohistochemical study on the...
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detection of the in vivo plasmin system in human breast pathology has been reported. Two recent morphological studies have been performed on the distribution of PA in colon tumors (15, 17) with some discordant results. Kohga et al. (17), using an immunoperoxidase technique, found UPA in all benign and malignant tumors with an intracellular labeling, whereas TPA was not associated with cancer cells. Burtin et al. (15), using a different purified antiserum against UPA free of contaminating antibodies against urinary proteins, detected UPA and TPA in malignant cells of only 12 and 14 tumors, respectively, in 34 colon carcinomas examined by immunofluorescence.

Our study, using the same antisera and the same methodology as Burtin et al. used, gives some analogous results. The first striking observation is the infrequent detection of UPA, as compared with previously reported data on breast carcinomas, and the presence of TPA in malignant cells of more than one-half of the cancers.

This finding may be the consequence of the action of PA inhibitors, characterized in macrophages (18) and fibroblasts (19). Tissot et al. (12) have also demonstrated the production of inhibitor(s) directed against UPA in extracts of breast carcinomas. UPA may also be present in amounts below the detection limit of the immunohistochemical method used.

However, the role of TPA present in many cancer cells in the production of plasmin and in the invasive process cannot be ruled out. TPA seems to be produced normally by mammary cells in vivo since it is seen in secretions, and this biosynthetic property may be preserved in malignant transformed cells. Besides, the presence of TPA without any associated PG or PAP in cancer cells of two carcinomas may be related to an early phase when PG or plasmin are not yet present or to a degradation of plasmin or may argue for a proteolytic action of TPA with substrates other than PG, as previously proposed by Quigley (20).

The detection of plasmin in its active form raises another problem since at the moment we have no antibody available against plasmin itself, only antisera against PG and PAP, the latter providing evidence for previous plasmin formation. Although PAP has a rather scanty distribution, PG has been found in 31 carcinomas regardless of their histological form and in only one benign lesion, an involuting lactating adenoma where tissue degradation histologically resembles that observed during malignant invasion. The presence of PAs, PG, and PAP in this lesion correlates with the immunocytochemical demonstration of UPA in epithelial cells of the involuting murine mammary glands (21). We must also emphasize the presence of both PG and PAP in the tubular carcinoma and their absence in the infiltrating epitheliosis as a useful tool for the differential diagnosis of those resembling proliferations.

Of particular interest is the localization of the components of the plasmin system in tumors. They are associated principally with invasive malignant cells. PG and PAP are detected frequently at the external contour of tumoral foci, unlike UPA and TPA, which are also found within tumoral clumps. Intraductal cancer cells rarely express all these markers. There is no close relationship between the loss of LM and the presence of PG, which seems to assign a limited role to plasmin in the breakdown of BM, the first step of tumor invasion. Plasmin is
tumor cells which are able to transform it in active plasmin through their PAs.

The plasmin system is obviously not the only proteinase system implicated in tumor invasion. Its role in the BM degradation in vivo remains to be specified, in light of our observations, but this morphological approach displays a close relation between the infiltration of the stroma by malignant mammary cells and this enzyme system.

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REFERENCES


Fig. 7. Noninvasive malignant lesion. LM is present within the BM with a disrupted pattern (arrow) whereas PG is not found in this zone. A, LM; B, PG; C, white light. x 400.
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