Cathepsin D and Dynamic Magnetic Resonance Imaging Gadolinium Enhancement in Malignant and Benign Breast Lesions

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ABSTRACT

Our purpose was to determine whether the expression of cathepsin D, a proteolytic enzyme implicated in basement membrane degradation, is associated with dynamic magnetic resonance imaging (MRI) enhancement of breast lesions. Forty-five patients with 48 breast lesions underwent gadolinium-enhanced spoiled gradient recalled echo MRI followed by excisional biopsy and cathepsin D staining and semiquantitative measurement in the lesions. There was no significant difference in cathepsin D staining of 25 malignant and 23 benign breast lesions. A significant association was seen between high cathepsin D staining and positive axillary lymph nodes in invasive carcinomas. Nine of nine (100%) node-positive carcinomas had high cathepsin D, as compared to three of seven (43%) node-negative carcinomas (P = 0.02). No significant associations were observed between cathepsin D staining and MRI enhancement amplitude, rate, or washout. Cathepsin D has no effect upon MRI gadolinium enhancement of malignant and benign breast lesions but is associated with positive axillary lymph nodes in invasive carcinomas.

INTRODUCTION

Cathepsin D has been purported to be a prognostic indicator in breast carcinoma (1-12). Cathepsin D is a proteolytic enzyme that has been implicated in the degradation of basement membranes. Gadolinium-enhanced breast MRI2 techniques are being studied to determine whether breast MRI may complement mammography in the detection of carcinoma or aid in the differentiation of benign from malignant lesions (13-25). Because delivery of intravascular agents to tumors requires transport of the agent across the vascular endothelium and basement membrane to the interstitium and cells of tumors, it is a reasonable hypothesis that cathepsin D levels in breast lesions may be associated with MRI contrast enhancement. We know of no studies in the literature that assess possible association between cathepsin D immunohistochemical staining and MRI enhancement of breast lesions. This study was undertaken to assess associations between cathepsin D staining of malignant and benign breast lesions and dynamic MRI parameters of amplitude, rate, and washout.

MATERIALS AND METHODS

This study population consists of a subgroup of 45 patients from a previously reported single-institution dynamic contrast-enhanced breast MRI trial (22), who underwent excisional biopsy and who also had cathepsin D staining of histological sections of each lesion. Each of the patients in this study (a) had a suspicious lesion detected by mammography and/or clinical examination, (b) volunteered and gave written informed consent for the Institute’s research review board-approved trial, (c) underwent dynamic contrast-enhanced MRI within 2 weeks of the diagnostic mammogram and clinical examination, and (d) underwent excisional biopsy of the lesion within 2 weeks of the breast MRI examination. The median age of the patients was 54 years (range, 26–82). Eighteen (40%) patients were 49 years or younger; 27 (60%) patients were 50 years or older.

The study population consisted of 26 lesions detected by mammography only, 13 lesions detected by mammography and clinical examination, and 9 lesions detected by clinical examination only in patients with normal mammograms. Twenty-six of the mammographic lesions were soft-tissue abnormalities; 13 were calcifications. The median size of the mammographic lesions was 13 mm (range, 5–60 mm). The median size of the clinically palpable lesions was 20 mm (range, 10–70 mm).

Breast MRI was obtained using a 1.5-T MRI clinical scanner (Signa; GE Medical Systems, Milwaukie, WI) and a commercially available GE breast coil (model no. M1085BR). This radio-frequency coil is a receive-only set of coils that permits the operator to select image acquisition from one or both breasts per data set. After axial localizer images were obtained, the involved breast was scanned in the sagittal plane using an 18-cm field of view, 3-mm section thickness, and 0-mm intersection gap. The T1-weighted images were obtained with a TR of 651 ms, TE of 12 ms, 256 × 192 matrix, and one acquisition. Fast spin-echo T2-weighted images were obtained with a TR of 6000 ms, TE of 120 ms, 256 × 128 matrix and two acquisitions. On the basis of the T1-weighted and T2-weighted images, five contiguous 3-mm sections were selected in the center of the lesion for SPGR pre- and postcontrast scanning using a TR...
Fig. 1  A, precontrast SPGR MR image. B, postcontrast SPGR MR image obtained 1 min after contrast injection shows enhancement of 2.5-cm-diameter mass (arrow). The amplitude of enhancement was 4.7 post-precontrast. Histological examination showed invasive ductal carcinoma. C, high-power photomicrograph (×400) shows dark intracytoplasmic staining of lysosomes for cathepsin D in both invasive carcinoma and DCIS. Cathepsin D staining was present in 20–50% of cells (2+). Axillary lymph node dissection was negative (0 of 10 nodes).
of 12.6 ms, TE of 3.6 ms, 60° flip angle, 256 × 192 matrix, and two acquisitions. Gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, NJ) was administered by hand as a bolus injection at a dose of 0.1 mmol/kg over 10 s followed by a 20-ml saline flush via extension tubing and a 20-gauge catheter in an antecubital vein while the patient was in the magnet, obviating the need for any patient movement between scans. Scanning began immediately following the injection, and serial dynamic images were obtained every 30 s for each of the five contiguous image sections over 5 min.

All breast MR images were interpreted jointly by three radiologists with knowledge of the mammographic and clinical findings but without knowledge of the histological findings. Matching film sets of pre- and postcontrast images were displayed and interpreted side by side. Two-mm² region of interest signal intensity measurements were made in two areas of maximal enhancement in the lesion as determined by visual inspection of the dynamic MRI scans. The region of interest showing the highest enhancement time-intensity curves. When tumor enhancement was not apparent by visual inspection of the MRI scans, lack of tumor enhancement in each case was confirmed by construction of computer-assisted pixel-by-pixel subtraction images. The three-parameter mathematical model used in earlier studies (20, 22) was fitted to the MR enhancement time-intensity curves using the equation:

\[ SI(t) = 1 + A[1 - \exp(-t/T_c)] - Ct \]

where \( SI(t) \) is the normalized signal intensity (signal intensity observed at time \( t \) divided by the precontrast signal intensity) at postcontrast time \( t \), \( A \) is the enhancement amplitude, \( T_c \) is the time constant for arrival of contrast material, and \( C \) is the first-order washout rate. Postcontrast/precontrast signal intensity ratios for each time interval during dynamic scanning were determined using the predicted curve.

**Histological Evaluation.** Histological material derived from excisional biopsy was stained with H&E. The histological interpretations were performed by one pathologist (J. S. W.) without knowledge of the MRI findings. For the purposes of this study, histological diagnoses were categorized as (a) invasive carcinoma including invasive ductal or invasive lobular carcinoma; (b) DCIS, including pure DCIS and DCIS with microscopic (<1 mm in diameter) foci of invasion, because it was uncertain whether the microscopic foci of invasion were included in the limited region of dynamic scanning; (c) fibroadenomas; or (d) other benign lesions.

**Cathepsin D Immunohistochemical Staining.** After review of each case, the most representative block of the suspicious lesion was selected. From each of the selected blocks, several consecutive 4-μm sections were obtained. One section was stained by immunohistochemical technique for cathepsin D antigen. Staining was performed on the Ventana 320 Automated Slide Staining System (Ventana Medical Systems, Tucson, AZ). The detection of cathepsin D utilized a mouse monoclonal antibody (Oncogene Science) at a dilution of 1:200 and the avidin-biotin complex technique following predigestion with protease. Diaminobenzidine was used as a chromogen, and the sections were then counterstained lightly with hematoxylin.

Cathepsin D staining was assessed by estimating the percentage of positively staining cells within each lesion examined. The amount of staining is indicated as 1+, 2+, or 3+, where 1+ represents fewer than 20% positive cells, 2+ represents 20–50% positive cells, and 3+ represents greater than 50% positive cells.

**Analysis.** To identify any association between the cathepsin D staining and MRI enhancement features, the following features were compared. For dynamic contrast-enhanced MRI, the following categorization of lesions with greater enhancement parameters was used: (a) greater amplitude: lesions that reached postcontrast/precontrast enhancement ratios equal to or greater than the median value of 3.0 within 2 min; (b) greater rate: lesions that had a time constant for arrival of contrast (parameter \( T_c \)) less than or equal to the median of 20; and (c) greater washout: lesions that during the 5-min dynamic scanning had a washout rate (parameter \( C \)) equal to or greater than the median of 1.0 × 10⁻³. Cathepsin D immunohistochemical staining of lesions was categorized as 3+ or 2+ or less.

The Fisher exact text was used to test the association in the 2 × 2 tables constructed using MRI enhancement amplitude (A) less than or equal to 3 versus amplitude greater than or equal to 3, amplitude less than or equal to 2 versus amplitude greater than or equal to 2, rate (\( T_c \)) greater than 20 versus rate (\( T_c \)) less than or equal to 20, or washout (\( C \)) less than or equal to 0.001 versus washout (\( C \)) greater than 0.001 (26). \( P \) values less than 0.05 were considered significant.

**Table 1** Cathepsin D staining of malignant and benign breast lesions

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>2+ or less</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>All lesions</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>All malignant lesions</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>DCIS</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>All benign lesions</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Fibroadenomas</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Other benign lesions</td>
<td>6</td>
<td>11</td>
</tr>
</tbody>
</table>

* There were no significant associations between cathepsin D staining and histological diagnosis.

- Two of three lobular carcinomas stained 3+; all others were ductal.

- Two cases had microscopic (less than 1 mm) foci of invasion.

**Table 2** Association between cathepsin D staining and size and nodal status of invasive carcinomas

<table>
<thead>
<tr>
<th>Pathological size</th>
<th>2+ or less</th>
<th>3+</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–10 mm</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>11–20 mm</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Greater than 20 mm</td>
<td>1</td>
<td>5</td>
<td>NS*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nodal status</th>
<th>2+ or less</th>
<th>3+</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative nodes</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Positive nodes</td>
<td>0</td>
<td>9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* NS, not significant.
RESULTS

Positive cathepsin D staining was noted in both benign and malignant epithelial cells and macrophages and was located in intracytoplasmic granules, most likely corresponding to lysosomes and endosomes (Fig. 1). In general, benign epithelium showed a low-intensity small granular staining, whereas in situ and invasive carcinomas and macrophages displayed a more consistent, diffuse, and higher-intensity staining of larger granules. Seventeen of 25 (68%) malignant and 17 of 23 (74%) benign lesions showed 3+ (greater than 50%) positivity staining cells for cathepsin D. These results are summarized in Table 1.

The associations between cathepsin D staining and size and nodal status of the invasive carcinomas included in the study is shown in Table 2. There was no significant association between cathepsin D staining and pathological size. There was a significant association between high cathepsin D staining and positive axillary lymph nodes. Of 16 patients who underwent lymph node dissection, 9 of 9 (100%) patients with positive lymph nodes had high (3+) cathepsin D staining of their primary tumors as compared to 3 of 7 (43%) patients with negative lymph nodes ($P = 0.02$).

The associations between MRI enhancement parameters and cathepsin D staining are shown in Table 3. No significant associations were observed between cathepsin D staining and the MRI enhancement parameters of amplitude greater or equal to three, amplitude greater or equal to two, rate, or washout.

DISCUSSION

Our study shows no significant association between cathepsin D staining and MR gadolinium enhancement amplitude, rate, or washout of malignant and benign breast lesions. However, a significant association was seen between cathepsin D staining and positive lymph nodes in invasive carcinomas. Nine of nine (100%) node-positive carcinomas had high cathepsin D as compared to three of seven (43%) node-negative carcinomas ($P = 0.02$).

Cathepsins are ubiquitous lysosomal enzymes that are classified both functionally and according to their active site. In the metastatic process, these proteolytic enzymes play a role in mediating passage of malignant cells between tissue and vessels via degradation of the basement membrane. As reviewed by Schwartz (2), measurements of cathepsin D in breast cancer have been shown in a majority of studies to be significant in predicting recurrence and may also predict disease-free and overall survival. A potential role of cathepsin D in models utilizing histological and molecular features of tumors in the hope of obtaining similar prognostic information as axillary lymph node dissection warrants continued investigation (27–29). Reported differences concerning the role of cathepsin D as a prognostic marker in breast cancer may be related in part to the methodology used and the employment of assays of antibodies prepared to different portions of the molecule (2, 4).

The use of MR enhancement parameters as an in vivo functional assay and predictor of biological and prognostic features of malignant and benign breast lesions has been a topic of several recent reports. In a prior report including 22 invasive carcinomas, we have shown no significant association between MR gadolinium enhancement parameters and tumor size, nodal status, or grade (22). An association between a peripheral pattern of enhancement in invasive carcinomas and high DNA S phase has been shown (23). Several studies of vessel density determined by factor VIII staining have shown that MRI gadolinium enhancement is not highly predictive of vessel density (24, 25).

Although basement membrane integrity would appear to play a role in any transport mechanism between vessels and surrounding tissue, its relative importance may depend on the size and transport mechanism for a given molecule or cell. Jain, describing barriers to drug delivery in solid tumors, has described multiple factors involving fluid and molecular transport in the delivery of intravascular agents through the endothelium and basement membrane into the interstitium and cells of lesions (30, 31). Macromolecular MRI contrast media currently being studied in animal models may be more suited than the low molecular weight gadolinium to define hyperpermeability associated with high cathepsin D levels (32–34). Further development of contrast-enhanced breast MRI and analysis techniques may also improve our ability to perform in vivo assays of vessel hyperpermeability in lesions.

We conclude that, although there is an association between high cathepsin D staining and positive axillary lymph nodes in invasive carcinomas, there is no significant association between...
cathepsin D staining and MR low molecular weight gadolinium enhancement amplitude, rate, or washout in malignant and benign breast lesions. Factors other than basement membrane integrity, including hemodynamics and capillary permeability and transport factors, are probably more responsible for the range of gadolinium enhancement seen in breast lesions.

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REFERENCES

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