Breast Epithelium Procurement from Stereotactic Core Biopsy Washings: Flow Cytometry-sorted Cell Count Analysis

Daniel L. Stoler, Carleton C. Stewart, and Paul C. Stomper

Departments of Experimental Pathology [D. L. S.], Flow Cytometry [C. C. S.], and Diagnostic Imaging [P. C. S.], Roswell Park Cancer Institute, Buffalo, New York 14263

ABSTRACT

Purpose: Molecular studies of breast lesions have been constrained by difficulties in procuring adequate tissues for analyses. Standard procedures are restricted to larger, palpable masses or the use of paraffin-embedded materials, precluding facile procurement of fresh specimens of early lesions. We describe a study to determine the yield and characteristics of sorted cell populations retrieved in core needle biopsy specimen rinses from a spectrum of breast lesions.

Experimental Design: Cells from 114 consecutive stereotactic core biopsies of mammographic lesions released into saline washes were submitted for flow cytometric analysis. For each specimen, epithelial cells were separated from stromal and blood tissue based on the presence of cytokeratin 8 and 18 markers. Epithelial cell yields based on pathological diagnoses of the biopsy specimen, patient age, and mammographic appearance of the lesion were determined.

Results: Biopsies containing malignant lesions yielded significantly higher numbers of cells than were obtained from benign lesion biopsies. Significantly greater cell counts were observed from lesions from women age 50 or above compared with those of younger women. Mammographic density surrounding the biopsy site, the mammographic appearance of the lesion, and the number of cores taken at the time of biopsy appeared to have little effect on the yield of epithelial cells.

Conclusions: We demonstrate the use of flow cytometric sorting of stereotactic core needle biopsy washes from lesions spanning the spectrum of breast pathology to obtain epithelial cells in sufficient numbers to meet the requirements of a variety of molecular and genetic analyses.

INTRODUCTION

Identification and characterization of breast tumor markers has important implications for breast cancer screening and diagnosis, for assessing prognosis, and for predicting response to individual therapies. In addition, determination of the molecular and/or genetic changes that precede or accompany the cancer can have significant effects on patient management and outcome. The greatest impact may be through defining these changes in early lesions that have been implicated in breast tumorigenesis, such as atypia, DCIS, and minimally invasive disease. However, a majority of these lesions are clinically occult and detected during screening mammography. Standard procedures for fresh tissue procurement are limited to larger, palpable masses, and the use of paraffin-embedded materials precludes the use of fresh tissues. Procurement of fresh epithelial cell specimens from earlier lesions poses unique challenges.

There will be more than 1 million breast biopsies in the United States alone this year, including an increasing proportion of percutaneous image-guided core biopsies of screening mammography-detected lesions (1). Stereotactic core needle biopsy of mammographically detected clinically occult lesions with a single-pass, vacuum-assisted biopsy needle provides a new source of raw material for research and clinical analyses. Cells can be recovered from washes of these specimens without sacrificing any histological material or requiring any added patient intervention (2, 3). Applying flow cytometry to these cell washes enables the separation of breast epithelial, stromal, and blood elements. Our initial experiences with the analysis of flow cytometry-sorted stereotactic breast core needle biopsies have been described (2, 3). We have shown that samples obtained by this procedure from malignant and benign tissues provide sufficient number of cells for analysis of aneuploidy by flow cytometry and for measurements of allelic imbalance in extracted DNA (3) of sorted cell populations. This report describes a prospective study of this technique to determine the yield and characteristics of sorted cell populations retrieved in core biopsy specimen rinses from a spectrum of lesions ranging from benign nonproliferative and proliferative lesions to early breast carcinoma.

MATERIALS AND METHODS

The study population consisted of 114 of 115 consecutive patients with clinically occult, mammographically suspicious lesions who underwent stereotactic core biopsy by one mammographer (P. S.) at the Roswell Park Cancer Institute mammography center. Following an explanation of the procedure and the nature of the research, all patients but one signed the

Received 10/10/01; accepted 11/15/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grant CNE-89517 from the American Cancer Society.

2 To whom requests for reprints should be addressed, at Department of Experimental Pathology, 501 Cell and Virus Building, Roswell Park Cancer Institute, Buffalo, NY 14263. Phone: (716) 845-8610; Fax: (716) 845-8126.

3 DCIS, ductal carcinoma in situ; FNA, fine-needle aspiration; CGH, comparative genomic hybridization.
informed consent prior to their biopsy. Patients’ ages ranged from 35 to 86 years (median, 56 years).

Stereotactic biopsy was performed using a stereotactic table with digital imaging (LoRad, Danbury, CT) and a directional vacuum-assisted biopsy device (Biopsy Medical, Irvine, CA) equipped with 14- or 11-gauge needles. This device allows extraction of several tissue cores with a single needle pass into the mammographic lesion, thus minimizing possible contamination from regions of the breast outside the area of the lesion. Five to >15 cores (median, 8) per biopsy were procured for each patient. Core specimens were initially placed in normal saline during the procedure and subsequently fixed in formalin and submitted for histological evaluation by Institute Department of Pathology staff. For those lesions associated with microcalcifications, core biopsy specimens were placed on films and radiographed to ensure adequate sampling (i.e., that the microcalcification was contained within the sample). Cells released into normal saline were sent to the Flow Cytometry Core Facility for cell sorting based on the expression of cytokeratins 8 and 18 and were sorted into cytokeratin-expressing (epithelial) and -nonexpressing cell populations as described below. Cytological evaluation of the core washings was not performed.

Prior to sorting, cells submitted for flow cytometric analysis were fixed in 2 ml of 70% ethanol and stored at −20°C until needed. Cells were collected by centrifugation at 1500 × g for 3 min, washed with 1 ml of PBS, and recentrifuged. FITC-conjugated antibodies specific for cytokeratins 8 and 18 were used as recommended by the manufacturer (Becton Dickinson Biosciences, San Jose, CA). The pellet was resuspended in 20 μl of antibody and incubated at 20°C for 30 min, diluted with 1 ml of PBS, and incubated an additional 10 min to allow unbound antibody to diffuse out of the cells. Cells were collected by centrifugation at 1500 × g for 3 min. The supernatant was removed, and the pellet was resuspended in 300 μl of PBS for sorting.

All measurements and cell sorting were performed using a FACSVantage flow cytometer (Becton Dickinson Biosciences). An air-cooled argon laser, operating at 488 nm, was used to excite the FITC, and instrument compensation was adjusted using normal blood gated on lymphocytes stained with FITC-conjugated CD45 or CD4PE antibodies in separate tubes. Logarithmic amplification was used to acquire all data. To ensure day-to-day consistency of measurements, a template defining the position of each cell type was created. Immunofluorescence of control cells was required to be ±40 channels of their mean at a resolution of 1024 each time a sort was performed. Instrument quality-assurance protocols and procedures have been previously reported (4).

All mammograms were prospectively interpreted by one mammographer (P. S.). For the purposes of this analysis, the mammographic appearance of the lesions was categorized as soft tissue masses or lesions manifested by microcalcifications only. The mammographic density of the breast parenchyma surrounding the lesion was categorized as dense (>90% dense tissue), fatty (>90% fatty tissue), or heterogeneous (11–89% dense tissue).

For the purposes of this study, malignant lesion pathological diagnoses were categorized as invasive carcinoma and DCIS. Benign lesion pathological diagnoses were categorized according to criteria described by Page et al. (5, 6) and used in similar analyses (7). Categories of pathological diagnoses of the benign lesions consisted of (a) atypical hyperplasia (benign tissue containing a focus or foci of atypical ductal or lobular hyperplasia); (b) hyperplasia (benign tissue containing a focus or foci of hyperplasia); (c) other proliferative changes (adenosis); and (d) nonproliferative changes, including fibroadenomas.

Statistical comparisons between groups were performed using the χ² test with P < 0.05 being the limit of statistical significance (8).

RESULTS

Flow cytometry-determined cell counts were obtained for 114 consecutive core needle biopsy washings. The pathological diagnoses for these lesions are shown in Table 1. Pathological diagnoses of the mammographically suspect lesions were approximately equally distributed among carcinomas, benign proliferative lesions, and benign nonproliferative lesions. Nearly half (49%) of the breast carcinomas were DCIS. Two-thirds (14 of 21) invasive carcinomas were minimal (≤10 mm) on pathological exam.

Cytokeratin-positive (epithelial) cells were detected in 108 of 114 (95%) patient specimens. The six cases (5%) where core biopsy washings contained only cytokeratin-negative cells consisted of two carcinomas and four benign lesions (Table 1). Only specimens that contained cytokeratin-positive cells were included in the subsequent analysis. The cell counts described represent the cytokeratin-positive (epithelial) cell population.

The distribution of cytokeratin-positive cell counts as well as their association with each lesion’s pathological diagnosis, patient age, and mammographic lesion appearance are shown in Table 2. The median cytokeratin-positive (epithelial) cell count for all specimens was 9,117 cells; however, cell counts of 200,000 or more could be obtained by this technique. Ninety-five percent (103 of 108) of the samples yielded >1,000 cytokeratin-positive cells, and approximately one-third of the samples yielded ≥20,000 cells.

There was a significant difference in cytokeratin-positive cell yield between the core biopsy washes of malignant and benign biopsy lesions. All carcinomas and 93% of benign le-

Table 1  Pathological diagnoses of stereotactic core biopsy specimens

<table>
<thead>
<tr>
<th>Pathological diagnoses</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin-positive specimens (n = 108; 95%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma (n = 41)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>DCIS</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Benign lesions (n = 67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypia/LCIS</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Other proliferative</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Nonproliferative</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Cytokeratin-negative specimens (n = 6; 5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Invasive</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>DCIS</td>
<td>4</td>
<td>66</td>
</tr>
</tbody>
</table>

*LCIS, lobular carcinoma in situ.
Breast Biopsy Epithelium Procured by Flow Cytometry

Although the number of the lesion. No increase was observed when increased number of cores taken within the area
of tissue. Rinses from biopsies of lesions within heterogeneous
tissue, and for rinses obtained from biopsies of lesions within dense tissue,
cells obtained in the rinses. The median cell counts were 9,138
rounding the lesion had a negligible impact on the number of
glandular epithelium relative to radiolucent fatty tissue sur-
or an increased proportion of radiodense stromal tissue and
the breast surrounding the core biopsy and the number of cores

We compared the yield of cells from biopsies where a mass was
found (Table 2). In contrast to previous findings using specimen mam-
mography-guided FNA biopsies, cytokeratin-positive cell counts
were significantly greater in samples from women age 50 or above.
The cell counts of specimens from patients below age 50 were on
average nearly 4-fold less than the counts from patients ≥50 years of age. Cytokeratin-positive cell counts >5000 were obtained from
84% (59 of 70) of samples from women ≥50 years of age compared with 40% (15 of 38) samples from patients <50 years of age
(P = 0.001).

Microcalcifications frequently represent the only mammog-
graphic evidence of many breast carcinomas and benign lesions. We compared the yield of cells from biopsies where a mass was not present but microcalcifications were detected with biopsies where a mammographically detectable mass was found (Table 2). No significant difference in cytokeratin-positive cell counts was found between these two groups.

Two potential influences on the yield of cells rinsed from the
tissue biopsies are the mammographic density of the area of
the breast surrounding the core biopsy and the number of cores that were taken. Increased mammographic density of the breast or an increased proportion of radiodense stromal tissue and glandular epithelium relative to radiolucent fatty tissue sur-
rounding the lesion had a negligible impact on the number of cells obtained in the rinses. The median cell counts were 9,138
for rinses obtained from biopsies of lesions within dense tissue, 8,143 for rinses from biopsies of lesions within fatty tissue, and
7,939 for rinses from biopsies of lesions within heterogeneous

tissue.

Intuitively, the core rinse cell yield would be expected to be increased with increased number of cores taken within the area
of the lesion. No increase was observed when 8–10 or 11–15
cores were extracted compared with 5–7. Although the number of
cytokeratin-positive cells obtained when >15 cores were
rinsed was lower than when fewer cores were used (Table 3),
only 3 such biopsies were performed, and this result probably reflects normal variation when small sample numbers are analyzed.

**DISCUSSION**

Standard fresh tissue procurement procedures for breast
specimens are limited to those lesions that are clinically palpable, narrowing the spectrum of acquired samples to relatively large invasive carcinomas and few DCIS. Non-
palpable minimally invasive and *in situ* breast carcinoma and benign lesions, such as atypias, hyperplasias, and other pro-
iferative lesions, are not often available for research pur-
poses. Molecular and biochemical analyses of these early malignancies and nonmalignant lesions hold great promise for identification of biomarkers for individuals with cancer or at high risk for cancer. In this report, we have demonstrated that the combination of stereotactic core biopsy washing procurement and flow cytometric sorting provides sufficient numbers of epithelial cells from lesions spanning the entire gamut of breast pathology to meet the requirements of a variety of experimental analyses (Table 4). Such techniques would include PCR-based methods, cytogenetic techniques, immunohistochemistry, and Western, Southern, and Northern blot analyses. Furthermore, this method requires no further patient intervention and does not hinder the histological
diagnosis made from the stereotactic core biopsy procedure. Research findings made from the cells, which otherwise are

---

**Table 2** Stereotactic core biopsy specimen cytokeratin-positive cell counts

<table>
<thead>
<tr>
<th>CKa positive cell count greater than</th>
<th>Total (n = 108)</th>
<th>Pathological diagnosis</th>
<th>Age (yrs)</th>
<th>Mammographic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malignant (n = 41)</td>
<td>Benign (n = 67)</td>
<td>n=49</td>
<td>n=50</td>
</tr>
<tr>
<td>1,000</td>
<td>103 (95%)</td>
<td>41 (100%)</td>
<td>62 (93%)</td>
<td>34 (90%)</td>
</tr>
<tr>
<td>2,000</td>
<td>86 (80%)</td>
<td>36 (88%)</td>
<td>50 (75%)</td>
<td>24 (63%)</td>
</tr>
<tr>
<td>5,000</td>
<td>74 (69%)</td>
<td>32 (78%)</td>
<td>38 (57%)</td>
<td>15 (40)</td>
</tr>
<tr>
<td>10,000</td>
<td>53 (49%)</td>
<td>26 (53)</td>
<td>23 (34)</td>
<td>8 (21)</td>
</tr>
<tr>
<td>20,000</td>
<td>35 (32)</td>
<td>20 (49)</td>
<td>14 (21)</td>
<td>6 (16)</td>
</tr>
<tr>
<td>50,000</td>
<td>18 (17)</td>
<td>12 (29)</td>
<td>5 (7)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>100,000</td>
<td>6 (6)</td>
<td>4 (10)</td>
<td>2 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>150,000</td>
<td>4 (4)</td>
<td>2 (5)</td>
<td>2 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>200,000</td>
<td>1 (1)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Median cell count</td>
<td>9,117</td>
<td>20,096</td>
<td>6,641</td>
<td>4,061</td>
</tr>
</tbody>
</table>

a CK, cytokeratin; Ca2+, calcification; w/o, without.  
b Percentages in parentheses.

---

**Table 3** Association between number of cores per biopsy and core wash cell counts

<table>
<thead>
<tr>
<th>Number of cores per biopsy</th>
<th>n (%)</th>
<th>Range of cytokeratin-positive cell count (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–7</td>
<td>26 (24)</td>
<td>987–195,761 (12,674)</td>
</tr>
<tr>
<td>8–10</td>
<td>51 (47)</td>
<td>670–217,277 (7,989)</td>
</tr>
<tr>
<td>11–15</td>
<td>28 (26)</td>
<td>744–173,029 (7,478)</td>
</tr>
<tr>
<td>&gt;15</td>
<td>3 (3)</td>
<td>1,553–9,849 (2,183)</td>
</tr>
</tbody>
</table>
The use of stereotactic core needle biopsies and flow cytometry for obtaining diagnostic data has been reported (13). However, in this case, entire cores were used to analyze DNA ploidy; thus the sample could not be evaluated histologically. Studies of breast ductal lavage have demonstrated that this technique can retrieve cytologically and molecularly aberrant epithelial cells in women at high risk for breast cancer or women with diagnosed cancer or DCIS (14, 15). Lavage may complement mammography screening for the detection of cancer when combined with mammography. However, ductal lavage requires additional patient intervention and cost, and cells retrieved by lavage cannot be localized to a specific site in the breast, limiting association of research findings with particular lesions.

Because multiple needle cores are obtained from the mammographic and pathological lesions, the surface or margin of whole or fragmented samples includes the lesion pathology as categorized as well as adjacent and intermixed epithelium and stroma. It is not known whether an underlying molecular or genetic change would be localized to a single epithelial pathological finding, the adjacent epithelium, or a field of breast tissue as well. These concepts are being investigated (16, 17). Increased cell marker specificity would require microdissection techniques or further refinement of flow cytometric sorting methods.

Using randomly selected PCR markers, we have previously demonstrated that the DNA extracted from sorted epithelial cells obtained from stereotactic core needle biopsy washes is suitable for the identification of loss of heterozygosity in benign breast lesions and early breast carcinomas (3). We are now exploring the use of DNA obtained from cells by this technique for the analysis of allelic loss in genes that have been implicated in breast tumor progression and genomic instability. We conclude that stereotactic core needle biopsy washes, which are otherwise routinely discarded by-products, provide adequate fresh breast epithelial cell samples for a considerable number of investigative molecular analyses. Flow cytometry can segregate epithelial cells, also serving as a screening test for adequate cytokeratin-positive cell counts for more efficient utilization in further molecular or genetic studies. The capability of analyses of the full spectrum of benign and early malignant lesions could lead to improved understanding of the nature of breast tumor progression.

ACKNOWLEDGMENTS

We thank Janice L. Hoffman and David L. Sheedy for outstanding technical assistance, Karin A. Brady for excellent secretarial skills, and Garth R. Anderson for valuable scientific discussions and input.

REFERENCES

Breast Epithelium Procurement from Stereotactic Core Biopsy Washings: Flow Cytometry-sorted Cell Count Analysis

Daniel L. Stoler, Carleton C. Stewart and Paul C. Stomper


Updated version  Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/8/2/428

Cited articles  This article cites 12 articles, 1 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/8/2/428.full#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.