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A Subset of Breast Invasive Ductal Carcinoma with Distinctive Cytomorphology, Aggressive Clinical Behavior, and Unique Immunologic Profiles

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BACKGROUND. Invasive ductal carcinoma of the breast is a heterogeneous collection of divergent types of carcinomas. Some subtypes have been characterized by histologic observations. This study describes a distinctive subset recognized through cytomorphologic examination of breast carcinoma specimens obtained by fine-needle aspiration biopsies (FNAB). Identification of this subset is established further by analyses of its clinical and immunologic characteristics.

METHODS. One hundred patients underwent FNAB and were diagnosed with breast ductal carcinoma. These diagnoses were followed by surgical resections and histologic evaluation of tumors. Immunohistochemical analyses of estrogen receptor, progesterone receptor, Her2/neu, p53 protein, and Ki-67 were performed. Patient's age, race, and family history of breast carcinoma were obtained. The objective of the study is to identify a cytomorphologically distinctive, clinically relevant, subset of breast carcinomas.

RESULTS. A subset carcinoma was recognized by cytomorphologic examination of Pap-stained FNAB slides. This subset consisted of seven patients with a median age of 37 years. At the time of surgical resection, all patients had axillary lymph node metastases. Six of seven patients had distant metastases. Immunohistochemical studies revealed that all tumors are positive for p53 protein and negative for estrogen and progesterone receptors.

CONCLUSION. This study presented a unique subset of breast ductal carcinomas that involved young patients and had aggressive growth behavior. These tumors expressed p53 protein but not estrogen and progesterone receptors. ***Cancer (Cancer Cytopathol)* 2002;96:294–300.** © *2002 American Cancer Society.*

Breast carcinoma is the most common cancer in women.[1] Invasive ductal carcinoma not otherwise specified (NOS) is the most common form, accounting for 80% of all breast carcinomas.[2,3] The term "ductal carcinoma" is used because these tumor cells exhibit variable degrees of histologic and cytologic differentiation toward mammary ductal epithelium.[3,4] However, it has long been recognized that the invasive ductal carcinoma, NOS, of the breast is a heterogeneous collection of carcinomas. Each carcinoma may have its own unique clinical, morphologic, and biologic characteristics.[5–7] Identification and characterization of these clinically relevant subsets of breast carcinomas could play a significant role in patient treatment and management.[8,9] Numerous efforts already have been made to subclassify these carcinomas based on tumor clinical behavior, immunologic and biologic markers, molecular profiles, and histologic observations.[8–11] So far, however, only limited success has been achieved relative to the urgent clinical need.

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In recent years, modern molecular technology, such as microarray technology, has generated great hope[12–14] and may be of value in characterizing these carcinomas in the future. Morphologic analysis of tumor cells, when used in combination with cellular and molecular studies, may still hold the key to understanding these issues. This study is an exploratory attempt to identify distinctive subsets of invasive breast ductal carcinomas through cytomorphologic observation of tumor cells. The validity of the identified subset and its clinical relevance are examined further by analyzing clinical characteristics, including patient age, TNM stage, and tumor cell immunohistochemical profile.

MATERIALS AND METHODS

One hundred cases of breast invasive ductal carcinoma initially diagnosed by fine-needle aspiration biopsies (FNAB) between 1996 and 2000 were obtained randomly from the Department of Pathology, Shands Hospital at Jacksonville, The University of Florida College of Medicine, Jacksonville, Florida. All FNABs were followed by surgical resections at the same hospital, and surgical specimens, biopsy or resection, were available for review. No diagnostic discrepancy was present between the FNABs and surgical pathology specimens.

Routine immunohistochemical profiles for breast carcinoma including expression of estrogen and progesterone receptor, p53, Ki-67, and Her2/neu were performed as part of a standard protocol for breast carcinoma evaluation in the clinical laboratory. Data were archived from files of pathology records. Clinical notes were not available for review. Each patient's pathology record, radiology record, and demographic information were reviewed. Tumor TNM status (tumor size, regional lymph node status, and distant metastasis) was evaluated based on information obtained from pathology reports. The presence of tumor distant metastasis was evaluated based on the information at the time of tumor workup and surgical resection. All cytology and histology slides were reviewed microscopically. Pap-stained slides of 100 FNABs were examined carefully and compared. Efforts were made to identify morphologically identical tumors by comparing cytologic appearances of tumor cells on Pap-stained FNAB slides, including the presence/absence of tumor cell pleomorphism, the size and shape of tumor cells, nucleus to cytoplasm (N/C) ratio, the texture of tumor cell cytoplasm, the intracellular location of nucleus, the size and shape of the nucleus, the characteristics of the nuclear membrane, the presence and size of the nucleolus, and the appearance of nuclear content. Seven cases were determined to have essentially identical appearance and were grouped as a subset. Age, TNM status, and immunohistochemical profiles of this subset were analyzed against the remaining cases. Statistical analyses were performed using the chi-square or Fisher exact test. All *P* values are two sided.

TABLE 1
Demographic Data of all Patients in the Study

All patients	100
Age (yrs)	
20–40	21
41–60	36
> 60	43
Family history	
Yes	8
No	92
Race	
White	38
Black	53
Other	9

TABLE 2
TNM Status of Tumors

All patients	100
Tumor size (cm)	
< 2	6
2–5	83
> 5	11
Axillary lymph nodes	
0	65
< 2	14
> 2	21
Distant metastasis	23
Histology grade	
I	1
II	24
III	75

RESULTS

Clinical Data

Demographic information is listed in Table 1. Patients ranged in age from 29 to 84 years. The median age was 62 years. The racial distribution was 38% white, 53% black, and 9% others. Eight percent of patients had first-degree relatives diagnosed with breast carcinoma.

TNM Status

The TNM status of all patients is summarized in Table 2. Tumor size ranged from 0.6 to 11 cm; the median tumor size was 3.2 cm. Metastatic axillary lymph nodes were found in 35% of patients. Among them, 14% had one to two positive lymph nodes and 21% had more than two positive lymph nodes. Twenty-

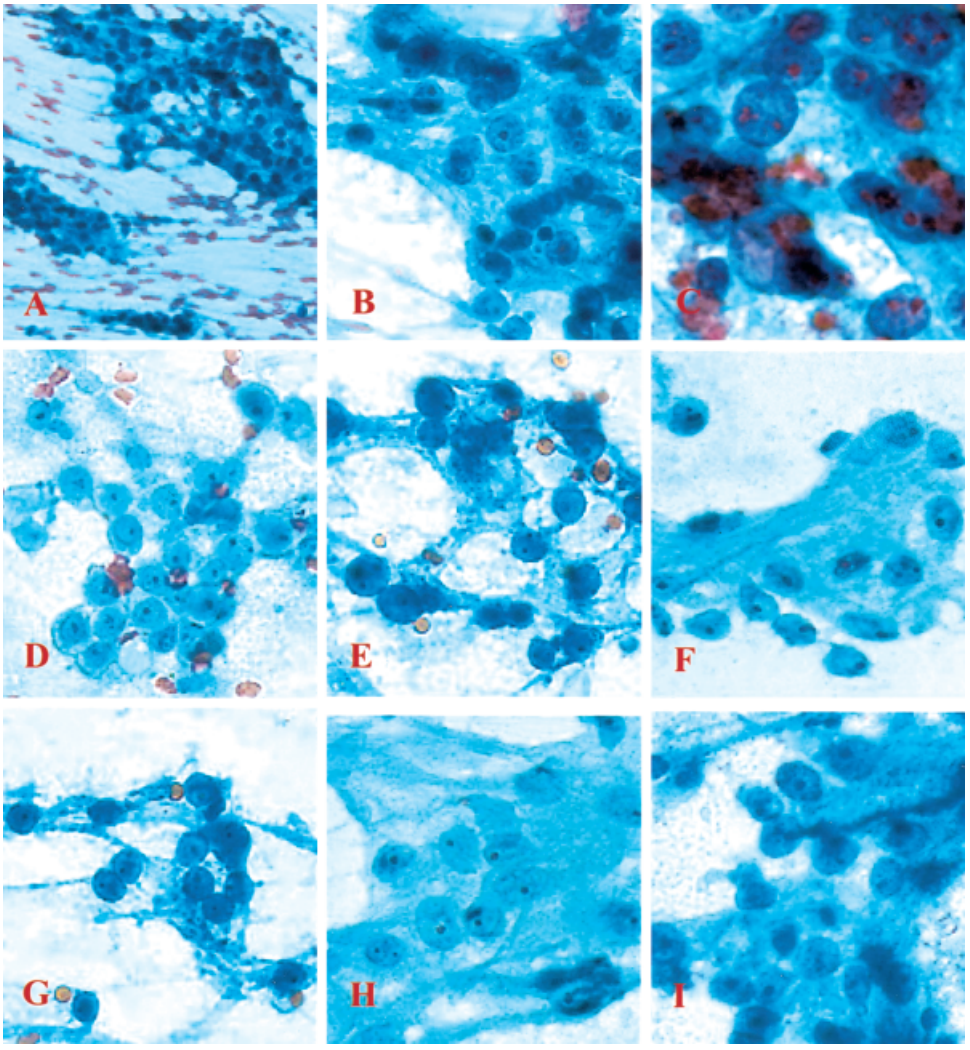

FIGURE 1. Cytomorphologic images of all seven invasive breast ductal carcinomas of the subset. Fine-needle aspiration biopsies were performed and the smears were prepared by Pap stains. (A) Photographic image of one case. × 40 magnification. (B) Photographic image of the same case. ×400 magnification. (C) Photographic image of the same case. ×1000 magnification. (D–I) Photographic images of the other six cases. ×400 magnification.

three of patients had distant tumor metastasis at the time of tumor resection, such as bone or brain metastasis. Seventy-five percent of patients had histologic high-grade carcinomas based on the surgical pathology report. These data indicated that these tumors were more advanced at time of initial diagnosis, which may reflect the characteristics of the patient population in our hospital.

Cytomorphologic Identification of a Distinctive Subset of Breast Carcinoma

Microscopic review of Pap-stained slides revealed a morphologically distinctive subset. It consisted of seven cases with essentially identical cytomorphologic features (the term "subset" will be used to represent this group of cases in the following text and figures). Figure 1 illustrates the primary morphologic features of this subset. At low magnification, a background of cellular debris was often present. Tumor cells were embedded in this background as loose clusters of uniform-appearing epithelial cells. In addition, there often were moderate to large numbers of singly dispersed cells. Some of the cytomorphologic features of tumor cells at high magnification included a delicate and vague cell membrane that often resulted in an ill-defined cell border, a loose and friable tumor cell cytoplasm that was often stripped off and left bare nuclei, an occasional well defined cytoplasmic vacuole, and perhaps the most distinctive feature, the appearance of nucleus and nucleoli of the tumor cells.

The most centrally located round nucleus had a uniform appearance. The nuclear membrane was smooth, although it was rigid and often thickened. A single prominent nucleolus sat in a slightly stained, either semitransparent or fine granular, nuclear background. Occasionally, irregular dark coarse nuclear chromatin elements were seen.

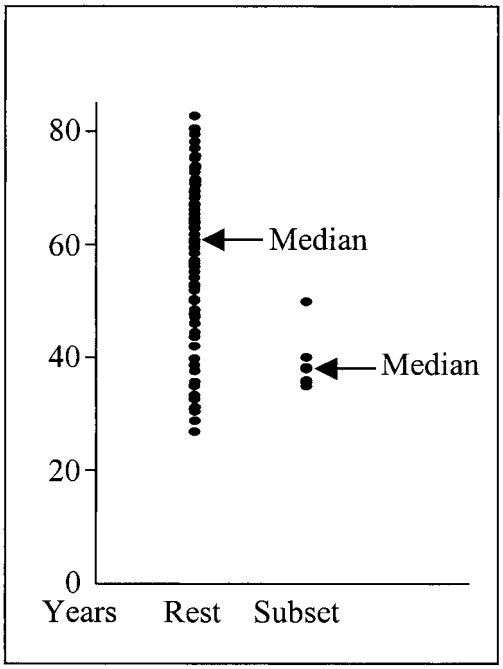

FIGURE 2. Age distributions of the subset and the remaining patients. Patients were divided into two groups, the subset (Subset) and the nonsubset (Rest) patients. Ages were plotted. Arrows indicate the median age of each group.

Additional Characteristics of the Subset

In addition to the distinctive morphologic features, the subset had unique clinical and immunologic characteristics compared with the remaining cases in the study. Patients were younger (median age, 37 years). The age of the subset ranged from 35 to 40 years, with one exception (the patient was 50 years old). In contrast, the remaining patients ranged in age from 29 to 83 years, with a median age of 62 years. These differences are statistically significant ($P < 0.05$; Fig. 2). All patients in the subset had metastatic carcinoma of the axillary lymph nodes (Fig. 3) and six of seven patients (86%) had more than two lymph nodes involved. Eighty-six percent (six of seven) of patients in the subset had distant metastatic carcinomas (Fig. 4). All tumors in the subset stained positive for p53 tumor suppressor protein and negative for estrogen receptor and progesterone receptor (Fig. 5).

No statistical differences were found between the subset and the remaining cases for family history of first-degree relatives diagnosed with breast carcinoma (Fig. 6); tumor size (Fig. 7); histology grade (Fig. 8); and Her2/neu and Ki-67 immunohistochemical stains (Fig. 5).

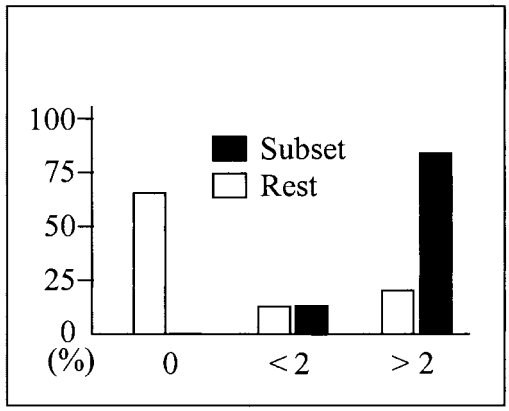

FIGURE 3. Occurrence of metastatic carcinoma of axillary lymph nodes. Patients of the subset (black bar) and rest (open bar) were divided into three groups; negative for metastasis (0), one to two lymph node metastases (< 2), and more than two lymph node metastases (> 2). Data of lymph node metastasis are illustrated as the percentage of occurrence.

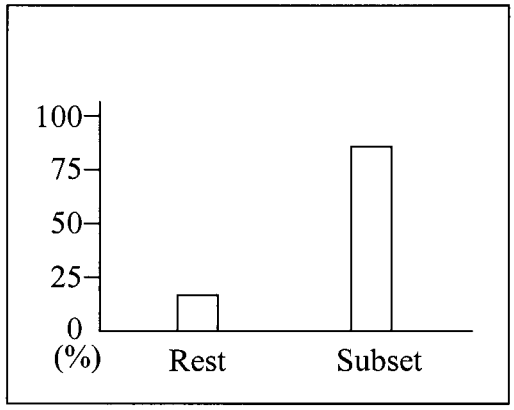

FIGURE 4. Occurrence of distant metastasis. Patients were divided into two groups, the subset (Subset) and the nonsubset (Rest). Distant metastasis is defined as the presence of tumors beyond the breast and axillary regions. Data of distant metastasis are illustrated as the percentage of occurrence.

Age-Matched Comparisons

Among 100 cases collected for this study, 21 patients (21%) were younger than 40 years of age, including 6 patients in the subset. Statistical comparisons indicate that non-subset young patients and patients older than 40 years of age shared the same clinical and immunohistochemical characteristics (Fig. 9). In contrast, young patients of the subset had marked higher incidences of nodal and distant metastases and p53 immunohistochemical positive stains (Fig. 9).

DISCUSSION

Breast invasive ductal carcinoma is a heterogeneous group of malignant neoplasms with different clinical and biologic characteristics.[3–7,15] Although various

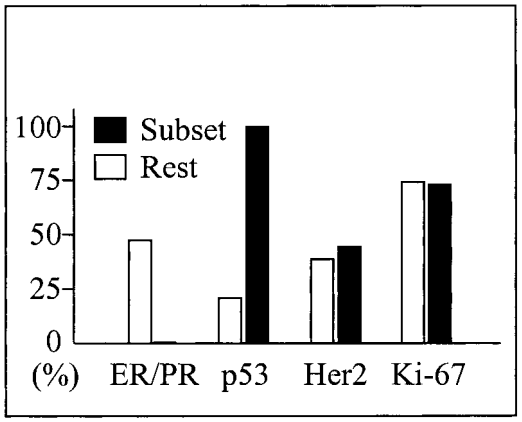

FIGURE 5. Immunohistochemical profiles of all tumors. Tumors of the subset (black bar) and rest (open bar) were analyzed for estrogen and progesterone receptors (ER/PR), p53 protein (p53), Her2/neu receptor (Her2), and Ki-67 protein (Ki-67) by immunohistochemical stains. Profiles were illustrated as the percentage of tumors stained positively.

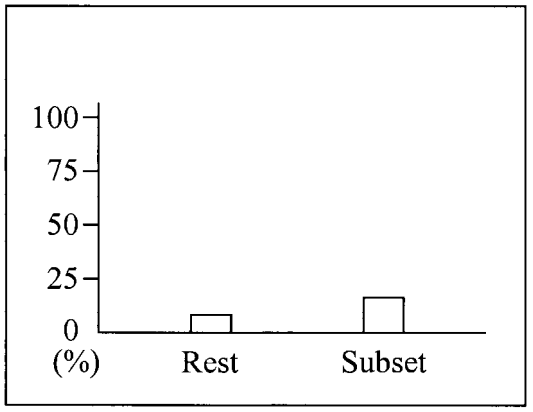

FIGURE 6. Presence of family history for breast carcinomas. Patients were divided into two groups, the subset (Subset) and the nonsubset (Rest). Family history of breast carcinoma is defined as the identification of breast carcinomas among first-degree relatives of the patients. Data are illustrated as the percentage of the presence of family history.

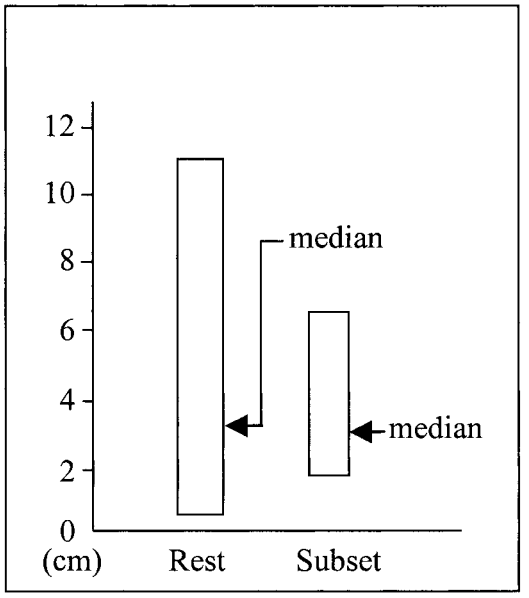

FIGURE 7. Tumor size distribution. Patients were divided into two groups, the subset (Subset) and the nonsubset (Rest). Tumor size data were provided by surgical pathology reports. Tumor sizes are expressed as maximum dimension in centimeters. Arrows indicate the median tumor size of each group.

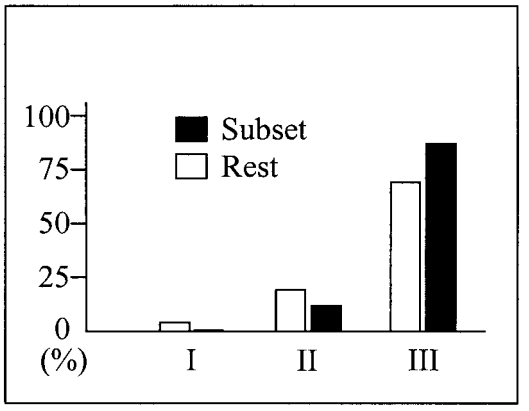

FIGURE 8. Distribution of tumor histology grade. Tumors of the subset (black bar) and rest (open bar) were graded using the Bloom-Richardson scale. Data on tumor grading were provided by surgical pathology reports. Tumors were divided into three groups: Grade I (I), Grade II (II), and Grade III (III). Distributions of tumor grades are expressed as the percentage presence of each grade.

morphologic appearances of breast ductal carcinomas are observed in routine surgical pathology practice, subclassification of these carcinomas has not been achieved.[16] However, a few rare variants of breast ductal carcinomas have been described morphologically, such as pleomorphic carcinoma,[17] secretory carcinoma,[18] and others.[19,20] The majority of breast ductal carcinomas are still nonclassifiable, or the NOS type.

As a result, a simple grading system was adopted.[21] This system, initially created in 1925 by Greenough[22] and modified in 1957 by Bloom and Richardson,[23] correlates well with a patient's prognosis.[24] However, this and modified systems used in cytology grading of breast carcinomas cannot predict the behavior of a given tumor. In fact, grading remains controversial because it may not add any value to the existing clinical staging as a prognostic indicator.[25] Currently, the clinical outcomes of patients with equivalent pathologic diagnoses vary widely.[15]

There is a genuine need for individualized evaluation of breast carcinoma beyond a pathologic three-level grading system.[26] Attempts have included all as-

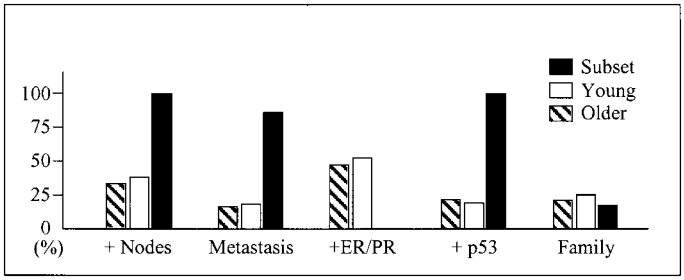

FIGURE 9. Age-matched comparisons. Of the 100 patients, 21 were 40 years old or younger. Of the 21 patients, 6 belonged to the subset (black bar). The subset was compared with the other 15 young patients (open box) and with the remaining older patients (striped box), in the following areas: presence of metastatic carcinoma of the axillary lymph nodes (+Nodes), presence of distant metastasis (Metastasis), positive immunohistochemical stains of estrogen and progesterone receptor (+ER/PR), positive immunohistochemical stains of p53 protein (p53), and presence of family history for breast carcinoma (Family). Data are illustrated as the percentage of the positive patients.

pects of the medical field. Clinically, family history and patient age became major factors.[27,28] Immunologic and biologic markers have become part of the standard evaluation of breast carcinomas, such as Her2/neu, p53, and Ki-67 protein expressions.[29–31] To our best knowledge, fine-needle aspiration cytology and cytomorphologic differentiation of breast ductal carcinoma NOS type have not been reported.

We believe that cytology in the evaluation of divergent breast carcinomas may have certain advantages over surgical histology. Because cellular appearance is the main focus of microscopic examination in cytology, the observations are obscured less by tissue architectures. Although numerous cytomorphologic appearances are seen commonly on cytologic examination of FNABs, the subset in this study appeared to stand on its own. This, as a morphologic observation, was supported by unique clinical and immunohistochemical profiles, such as young age, aggressive behavior, negative immunohistochemical stains for estrogen and progesterone receptors, and positive stains for p53.

These observations may have provided additional information regarding breast carcinomas in young patients. Studies in the literature found that breast carcinomas in young patients overall tend to be more aggressive, have a higher probability of recurrence, and have a high rate of both lymph node and distant tumor metastases.[32,33] These tumors statistically tend to be p53 positive and estrogen and progesterone receptor negative by immunohistochemical stains.[32–34] The results of this study showed that the identified carcinoma subset occurred predominantly in young patients, except for one (Fig. 2). These tumors were highly aggressive and featured lymph node and distant metastases (Figs. 3 and 4). They had immunohistochemical profiles that were negative for estrogen and progesterone receptors and positive for p53 tumor suppressor protein (Fig. 5). Statistical analyses further indicated that after excluding the subset, the remaining tumors of young patients (40 years old or younger) were not statistically different to those of older patients in the study (Fig. 9). These facts suggest that the unique tumor characteristics of the young patients described in the literature may account for the characteristics of this subset.

Reviewing the histology of these tumors also indicated that their cytomorphologic features are very similar. A thorough histology study is needed to further characterize these tumors. The value of histology in identifying this subset remains uncertain at this time.

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