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# Histological and Immunohistochemical Evaluation of Ductal Carcinoma *In Situ* Co-Existing with Triple-Negative Carcinoma of the Breast

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**Background :** Triple-negative breast carcinomas (TNBCs) are associated with high-grade histological tumor and a poor clinical outcome. In this study, we evaluated the histology and immunohistochemical features of DCIS co-existing with TNBC to determine the characteristics of the precursor lesions of TNBC. **Methods :** Among the 1,610 cases of breast carcinoma, we selected the TNBCs with DCIS (n=196), and compared the pathological and immunohistochemical findings of the DCIS with those of the invasive carcinoma areas. **Results :** Among the 1,610 breast carcinomas, the TNBCs accounted for 330 cases (20.5%) and there were 196 cases with DCIS. The TN-DCIS cases exhibited high nuclear (94.5%) and histological (94.5%) grades, comedo-necrosis (68.9%) and a small extent of the DCIS-involved area. Immunohistochemically, a p53 expression was present in 48.4% of the TN-DCIS cases and a high Ki-67 index was present in 31.5%. The same TN immunohistochemical profiles as the carcinoma were detected in 109 of the 124 (87.9%) cases, but different profiles were observed in 15 of the 124 (12.1%) cases. The 15 discordant cases were associated with a low histological grade (p=0.037), low p53-positivity (p=0.006) and a low Ki-67 index (p=0.026), as compared to the invasive carcinomas. **Conclusions :** The results of this study suggest that TN DCIS is a highly probable, but not obligate, precursor lesion of TNBC.

**Key Words :** Breast; Noninfiltrating intraductal carcinoma; Immunohistochemistry

Human breast cancer is a heterogeneous disease that shows a significant amount of biological and histological diversity. Based on the classification of the tumors by the gene expression patterns derived from cDNA microarray assays, a hierarchical clustering has been demonstrated for five major biologically distinct subtypes; these subtypes are associated with significant differences of the clinical outcome and these subtypes include: luminal A, luminal B, human epidermal growth factor receptor-2 (HER2) overexpressing, basal-like and normal breast-like.[1-3] Within these subtypes, HER2 overexpressing and basal-like breast cancers have the worst prognosis as compared to the other subtypes.[2,3] Immunohistochemical surrogates for breast cancer subtyping have been developed and used in the clinical setting[4-7] and they include the estrogen receptor (ER), progesterone receptor (PR), HER2, epidermal growth factor receptor-1 (EGFR), c-KIT, basal (CK5/6, CK14, and CK17) and luminal (CK7/8, CK18, and CK19) cytokeratins (CK).[8]

Among the five subtypes, the basal-like subtype has been characterized by cDNA microarray profiling by a high expression of the genes that are associated with breast basal epithelial cells, and the basal-like subtype is negative for ER, PR and HER2 (the so-called 'triple negative phenotype') and positive for basal CK, EGFR, and/or c-KIT, as determined by immunohistochemical analyses. The basal-like subtype and triple-negative breast cancer (TNBC) are not the same entity;[9] however, most basal-like subgroups are included in the TNBC category. Therefore, the triple-negative (TN) phenotype is commonly used as a clinical surrogate for basal-like tumors.[10,11] Because there is no expression of hormonal receptors (ER and PR) and HER2, these basal-like tumors do not benefit from specific targeted therapy such as hormonal treatment and trastuzumab administration.

Ductal carcinoma *in situ* (DCIS) is generally regarded as a precancerous lesion that's associated with the development of invasive carcinoma. The association of TN with DCIS has been reported in a few studies that have demonstrated the existence of a pure DCIS with the basal phenotype[12,13] and also a few cases of DCIS that co-exist with TNBC. This suggests the possibility of a precursor lesion that might be associated with the development of TNBC.[14]

In this study, we classified the breast carcinomas of Korean women into four subtypes with using the triple markers ER, PR and HER2, and we determined the prevalence of each subtype and its association with DCIS. After the co-existence of DCIS and TNBC was confirmed, we evaluated the histological and immunohistochemical features of the DCIS co-existing with the TNBC to determine the characteristic features of the precursor lesions that are associated with TNBC.

## MATERIALS AND METHODS

### Case selection

A total of 1,610 cases of breast carcinomas were collected by reviewing the pathology reports of the patients who underwent surgical breast resection at Seoul National University Hospital from 2006 through 2007. In addition, the histological diagnosis, the nuclear and histological grades of invasive carcinomas as assessed by a modified Bloom-Richardson system and the presence/absence of DCIS were confirmed. All of the enrolled patients were Korean women, and their mean age at surgery was 48.13 years (range of age 20-83 years). The collected breast carcinomas were classified into four subtypes (Luminal, Luminal/HER2, HER2 and Triple-negative) according to the ER, PR and HER2 expression status of the invasive tumor, based on the immunohistochemical profiles at the time of the diagnosis: Luminal (ER+ and/or PR+, HER2-), Luminal/HER2 (ER+ and/or PR+, HER2+), HER2 (ER- and PR-, HER2+), and Triple-negative (ER- and PR-, HER2-). The TN subtypes were identified in 330 cases out of the total 1,610 cases. Among these TN subtypes, the cases with DCIS (196 of 330 cases) were evaluated for their histological and immunohistochemical features.

### Histological evaluation of DCIS co-existing with TNBCs

Among 196 cases of TNBC with co-existing DCIS, the histological features of the DCIS were reviewed in 164 cases. Thirty cases were excluded due to having received preoperative chemotherapy and two cases were unavailable. The tumors, including DCIS, were consecutively embedded in paraffin at 5 mm intervals for making measurements of the size of the invasive carcinomas and the extent of the DCIS. The formalin-fixed, paraffin-embedded tissue blocks were cut in 4 $\mu$m sections, and then they were stained with hematoxylin and eosin. The nuclear grade of the DCIS was assessed as two grades (low or high), based on the nuclear size and regularity, the chromatin pattern, the number and size of the nucleoli and the mitotic activity. The histological grade of the DCIS was divided into three categories according to the nuclear grade in combination with the presence/absence of necrosis (according to the "Van Nuys pathologic classification"): low (a low nuclear grade with no necrosis), intermediate (a low nuclear grade with necrosis) and high (a high nuclear grade with or without necrosis). The distribution of the DCIS was classified into three categories: present within the tumor and mixed with invasive carcinoma, present around the tumor and present with both. The percentage of the DCIS in the total tumor volume was estimated for the cases with the DCIS present within the invasive carcinoma. When the DCIS was present around the invasive carcinoma, the difference in the greatest dimension between the invasive tumor only and the tumor with the DCIS as well as the invasive carcinoma was used to calculate the extent of the DCIS. This was then divided into three sections: less than 1 cm, 1 cm or more and less than 2 cm, and 2 cm or more. Other histological features such as presence/absence of comedo-necrosis and microcalcification were also evaluated.

### Immunohistochemical evaluation of DCIS co-existing with TNBCs

Immunohistochemical staining for ER, PR, HER2, p53 and Ki-67 was routinely performed in all the breast carcinomas using an automated immunostainer (Techmate 500 plus, DAKO A/S, Copenhagen, Denmark) at the time of the diagnosis. The information on the primary antibodies used in this study is listed in Table 1. We used 3,3′-diaminobenzidine tetrahydrochloride (DAB) for visualization of antibody/enzyme complexes and we counterstained the samples with Mayer's hematoxylin. Among 196 cases of TNBC with co-existing DCIS, immunohistochemical re-examination was performed in the 124 available cases.

For ER and PR, the receptor positive results were defined as nuclear staining in 10% or more of the tumor cells. The HER2 overexpression was considered as moderate to strong membra-

Table 1. Information of primary antibodies used in this study

| Antibody | Clone | Manufacturer | Dilution | Antigen retrieval |
|---|---|---|---|---|
| ER | 1D5 | DakoCytomation | 1:50 | Microwave oven |
| PR | PgR636 | DakoCytomation | 1:50 | Microwave oven |
| HER2 | CB11 | Novocastra | 1:200 | Microwave oven |
| p53 | DO7 | DakoCytomation | 1:800 | Microwave oven |
| Ki-67 | MIB-1 | DakoCytomation | 1:1,000 | Microwave oven |

nous staining in greater than 10% of the tumor cells, which was equivalent to a score of 2+ or 3+ by the HercepTest protocol.[15] The p53 expression was graded into five groups according to the proportion of tumor cells with nuclear p53 staining as no expression, positive in less than 25%, positive in 25% or more and less than 50%, positive in 50% or more and less than 75%, and positive in more than 75%; a positive p53 expression was defined as positive results of 25% or higher. A high Ki-67 index was defined as nuclear staining in more than 10% of the tumor cells.

### Statistical analysis

The $\chi^2$ test and Fisher's exact test were used to compare frequencies in the contingency tables. A bivariate correlation analysis was used to compare the p53 and Ki-67 indexes of the carcinomas with those of the co-existing DCIS, and Pearson's correlation coefficient (r) was calculated for the p53 and Ki-67 index between the carcinomas and the co-existing DCIS. A p-value of $\leq 0.05$ was considered to be statistically significant. All the statistical analyses were carried out using SPSS 12.0 for Windows (SPSS, Inc.).

## RESULTS

### Grouping of the breast carcinomas by the immunohistochemical subtype using triple markers

The majority of the histology diagnoses were invasive ductal carcinomas, not otherwise specified (NOS) (1412, 87.7%), followed by microinvasive carcinomas (41, 2.5%), invasive lobular carcinomas (35, 2.2%), mixed invasive ductal and lobular carcinomas (34, 2.1%), and mucinous carcinomas (31, 1.9%). The categorization of the breast cancer and the frequency of DCIS for each subtype are summarized in Table 2. TNBCs were present in 330 of the 1,610 cases (20.55%), and co-existing DCIS was observed in 196 out of the 330 cases (59.4% within the subtype). Co-existence of DCIS was frequently noted in the HER2-positive group, including the luminal/HER2 and HER2 subtypes (88.9% and 86.7% within the subtypes, respectively), and this was relatively uncommon in the TN subtype ($p<0.001$).

Table 2. Prevalence of breast carcinoma and co-existing DCIS

| Subtype | Number of carcinoma (n=1,610) (% within total breast cancer) | Number of carcinoma with co-existing DCIS (n=1,225) (% within subtype) |
|---|---|---|
| Luminal | 860 (53.4) | 660 (76.7) |
| Luminal/HER2 | 217 (13.5) | 193 (88.9) |
| HER2 | 203 (12.6) | 176 (86.7) |
| Triple negative | 330 (20.5) | 196 (59.4) |

About half of the microinvasive carcinomas (21 cases) were included in the HER2 subtype, and this was three times as many compared to the other subtypes. Most of the invasive lobular carcinomas, mixed invasive ductal and lobular carcinomas, mucinous carcinomas and tubular carcinomas were the luminal subtype; the metaplastic carcinomas and medullary carcinomas were the TN subtype (Table 3).

### Histological features of DCIS co-existing withTNBCs

Most of the DCIS co-existing with the TNBC cases showed high grade features for the nuclear (155, 94.5%) and histological (155, 94.5%) grades (Fig. 1A, B). Among the 9 DCIS with a low nuclear grade, 8 (88.9%) co-existed with invasive carcinoma with a nuclear grade of 2, and only one case (11.1%) had a nuclear grade of 3. On the other hand, the majority of the DCIS with a high nuclear grade co-existed with grade 3 invasive carcinomas (143 cases, 92.3%). Similarly, only 2 out of 9 (22.2%) histological low-grade DCIS were accompanied with histological grade 3 invasive carcinoma, and 137 out of 155 (88.4%) histological high-grade DCIS were accompanied with

Table 3. Prevalence of histologic diagnoses of breast carcinomas according to subtypes

| Subtype | Subtype (% within diagnosis) | | | | Total (n=1,610) (% total) |
|---|---|---|---|---|---|
| | L | L/H | H | TN | |
| IDC | 741 (52.5) | 197 (14.0) | 174 (12.3) | 300 (21.2) | 1,412 (87.7) |
| MIC | 7 (17.1) | 7 (17.1) | 21 (51.2) | 6 (14.6) | 41 (2.5) |
| ILC | 33 (94.3) | 0 | 0 | 2 (5.7) | 35 (2.2) |
| MDLC | 31 (91.2) | 3 (8.8) | 0 | 0 | 34 (2.1) |
| MucC | 28 (90.3) | 2 (6.5) | 0 | 1 (3.2) | 31 (1.9) |
| IMPC | 2 (13.3) | 7 (46.7) | 4 (26.7) | 2 (13.3) | 15 (0.9) |
| MetC | 0 | 0 | 1 (8.3) | 11 (91.7) | 12 (0.7) |
| TC | 10 (100) | 0 | 0 | 0 | 10 (0.6) |
| IPC | 5 (83.3) | 1 (16.7) | 0 | 0 | 6 (0.4) |
| MedC | 0 | 0 | 0 | 5 (100) | 5 (0.3) |
| ApoC | 0 | 0 | 3 (75.0) | 1 (25.0) | 4 (0.2) |
| Miscellaneous | 3 (60.0) | 0 | 0 | 2 (40.2) | 5 (0.3) |

L, luminal; L/H, luminal/HER2; H, HER2; TN, triple-negative; IDC, invasive ductal carcinoma; MIC, microinvasive carcinoma; ILC, invasive lobular carcinoma; MDLC, mixed ductal and lobular carcinoma; MucC, mucinous carcinoma; IMPC, invasive micropapillary carcinoma; MetC, metaplastic carcinoma; TC, tubular carcinoma; IPC, invasive papillary carcinoma; MedC, medullary carcinoma; ApoC, apocrine carcinoma.

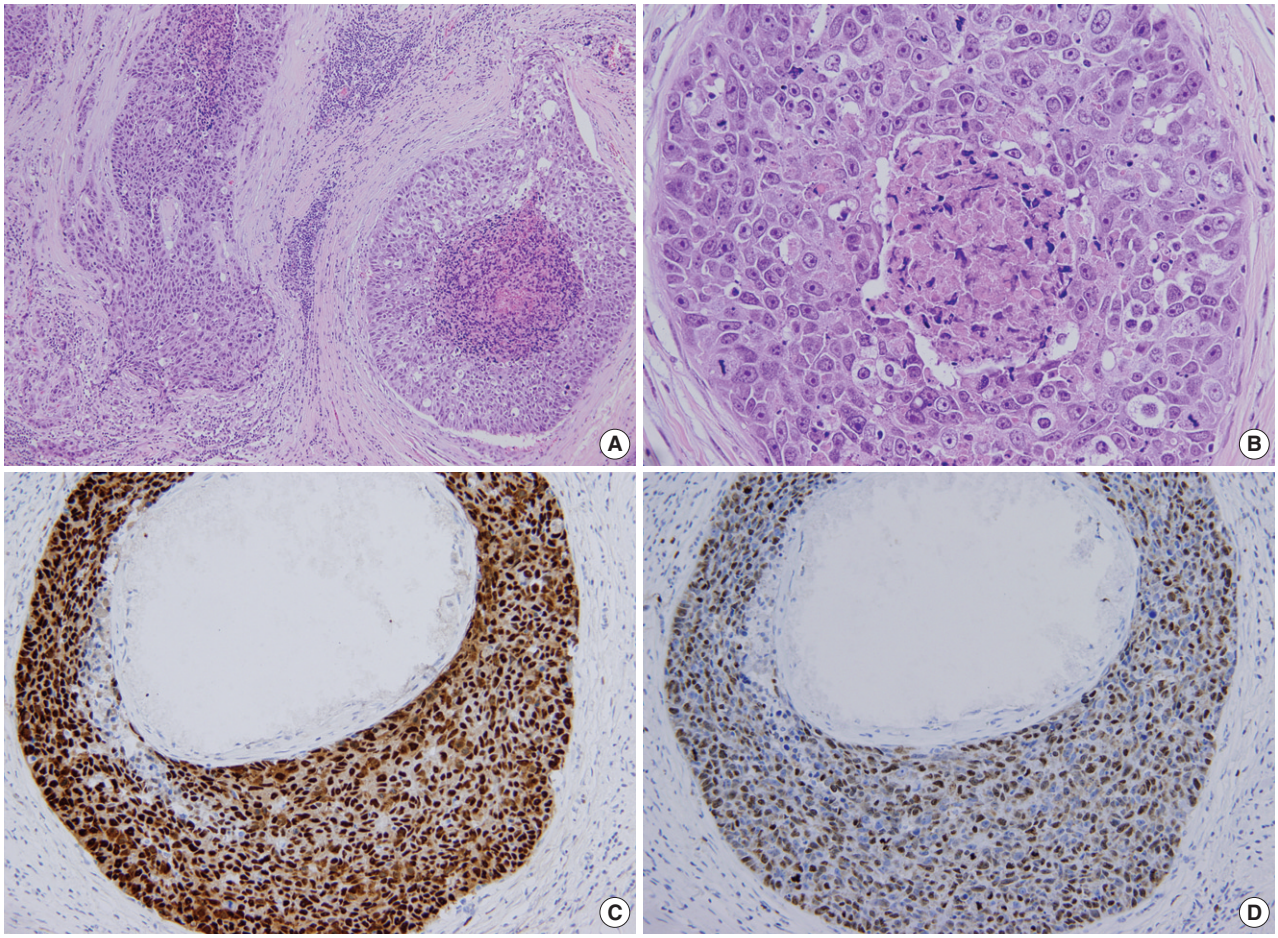


Fig. 1. (A) DCIS co-existing with triple-negative breast carcinoma is identified around the invasive triple-negative breast carcinoma. (B) This DCIS shows high grade nuclear features and comedo-necrosis. (C) p53 is highly expressed in the DCIS (nearly 100%). (D) Ki-67 index is about 40% in the DCIS.

histological grade 3 invasive carcinoma. Comedo-necrosis was noted in 135 of 196 (68.9%) cases and this was found only in the cases that exhibited a high nuclear grade; an intermediate histological grade was not present in these cases. The majority of the co-existing DCIS cases were distributed around invasive tumors (103, 62.8%), some were within the tumor and mixed with the invasive carcinoma (33, 20.1%), and the remainder (28, 17.1%) were distributed both around and mixed with the invasive tumors. When the DCIS samples were noted to be within the invasive tumor (n=61), a number of DCIS cases (40, 65.6%) occupied less than 5% of the total tumor volume, and more than 90% of the DCIS cases occupied less than 25% of the total tumor volume. When the DCIS cases were identified around the invasive tumors (n=131), the difference in the greatest dimension of the tumors, between invasive carcinoma only and the invasive carcinoma and the DCIS, was calculated as described above; it was less than 1 cm in 36 (48.1%) cases and 2 cm or more in 36 (27.5%) cases. Microcalcifications of the DCIS were identified in 46 (28.4%) cases, and this was associated with the presence of comedo-necrosis (p=0.008) and a high histological grade (p=0.012).

### Immunohistochemical comparison of DCIS co-existing with TNBCs

p53 overexpression of DCIS was found in 60 of 124 (48.4%) cases, and a high Ki-67 index was found in 39 of 124 (31.5%) cases (Fig. 1C, D). The p53 expression of co-existing DCIS strongly correlated with that of the invasive carcinoma (r=0.968, p<0.001). In addition, the Ki-67 index of the DCIS and that of the invasive carcinoma were also correlated (r=0.809, p<0.001). The immunohistochemical TN phenotype, which was identical to the corresponding carcinomas, was noted in most of the DCIS (109, 87.9%). However, different phenotypes were found

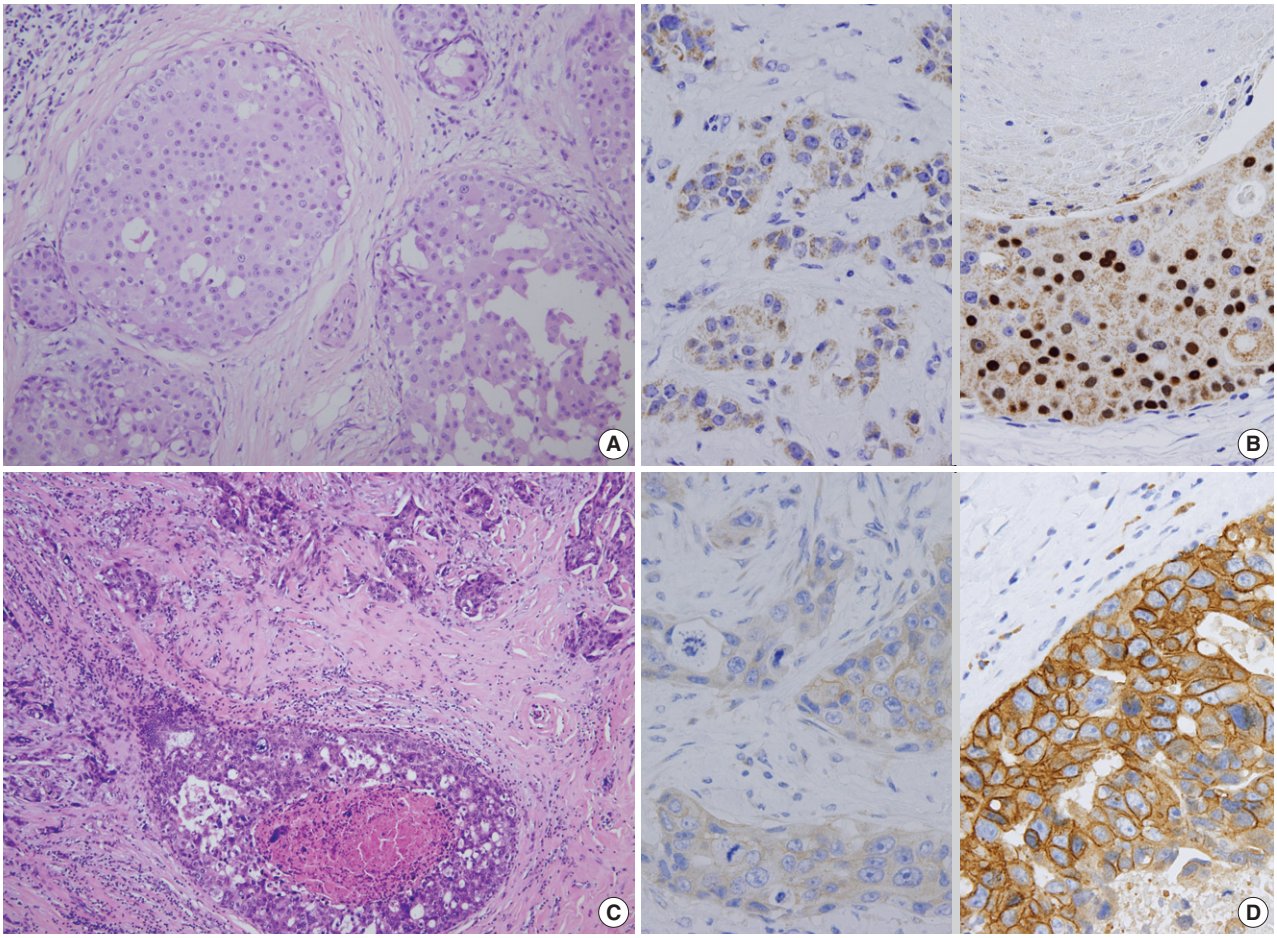


Fig. 2. (A) The DCIS with triple negative carcinoma shows high-grade histology. (B) Nuclear staining of ER is negative in the triple negative invasive carcinoma with weak cytoplasm staining (left), but strong positive results in the co-existing DCIS (right). (C) High grade DCIS around the tumor shows comedo-necrosis. (D) HER2 staining is negative in the triple negative invasive carcinoma (left), but moderate to strong membranous staining of the HER2 is noted in the DCIS (right).

in 15 cases; 12 had a luminal subtype (Fig. 2A, B) and three a HER2 subtype (Fig. 2C, D). On comparison of the immunohistochemical concordant cases with the discordant cases (Table 4), a low histological grade of the co-existing TNBC was more commonly observed in the discordant cases than that in the concordant cases (33.3% vs 11.0%, respectively, p=0.037). However, there was no significant difference observed in the histological grade of the DCIS between the discordant and concordant cases (p=0.481). On the other hand, the discordant cases were significantly associated with low p53-positive results (86.7%, p=0.006) and a low Ki-67 index (86.7%, p=0.026) for the co-existing carcinomas, and there was a tendency for low p53-positivity (73.3%, p=0.099) and a low Ki-67 index (86.7%, p=0.142) for DCIS, with a marginal significance. A large extent of the DCIS and the presence of microcalcification were observed more frequently in the discordant cases (p=0.010 and 0.001, respectively) than that in the concordant cases.

## DISCUSSION

The recent studies that used cDNA microarray profiling have demonstrated five distinctive subtypes of breast cancer that have significant differences in their immunophenotype and clinical outcomes.[1-5] Among these five subtypes, the basal-like subtype is associated with a larger size, a higher histological grade, pushing margins, a poorer Nottingham Prognostic Index, the development of disease recurrence and distant metastasis,[10,16,17] as well as having a p53 expression.[5,7]

The basal-like subtype accounts for 17-37% of all breast cancers according to the classification based on cDNA microarray profiling.[3] Although there are some differences of the immuno-

Table 4. Comparison between immunohistochemical concordant and discordant cases

| Characteristic | No. (%) | | p-value |
|---|---|---|---|
| | Concordant (n=109) | Discordant (n=15) | |
| Nuclear grade of carcinoma | | | |
| 2 | 11 (10.1) | 4 (26.7) | 0.085[a] |
| 3 | 98 (89.9) | 11 (73.3) | |
| Histological grade of carcinoma | | | |
| 1 or 2 | 12 (11.0) | 5 (33.3) | 0.037[a] |
| 3 | 94 (86.2) | 10 (66.7) | |
| Unknown | 3 (2.8) | 0 | |
| p53 positivity of carcinoma | | | |
| ≥25% | 56 (51.4) | 2 (13.3) | 0.006[a] |
| <25% | 53 (48.6) | 13 (86.7) | |
| Ki-67 index of carcinoma | | | |
| ≥10% | 48 (44.0) | 2 (13.3) | 0.026[a] |
| <10% | 61 (56.0) | 13 (86.7) | |
| Nuclear grade of DCIS | | | |
| Low | 4 (3.7) | 1 (6.7) | 0.481[a] |
| High | 105 (96.3) | 14 (93.3) | |
| Histological grade of DCIS | | | |
| Low | 4 (3.7) | 1 (6.7) | 0.481[a] |
| High | 105 (96.3) | 14 (93.3) | |
| p53 positivity of DCIS | | | |
| ≥25% | 56 (51.4) | 4 (26.7) | 0.099[a] |
| <25% | 53 (48.6) | 11 (73.3) | |
| Ki-67 index of DCIS | | | |
| ≥10% | 37 (33.9) | 2 (13.3) | 0.142[a] |
| <10% | 72 (66.1) | 13 (86.7) | |
| Extent of DCIS | | | |
| <1 cm | 41 (37.6) | 1 (6.7) | 0.010[b] |
| 1 cm and <2 cm | 18 (16.6) | 6 (40.0) | |
| ≥2 cm | 25 (22.9) | 8 (53.3) | |
| Undetermined | 25 (22.9) | 0 | |
| Microcalcification of DCIS | | | |
| Present | 29 (26.6) | 11 (73.3) | 0.001[a] |
| Absent | 80 (73.4) | 4 (26.7) | |

[a], Fisher's exact test; [b], $\chi^2$ test.

histochemical definitions of the subtypes and the subjects included in each study, many studies using an immunohistochemical classification of breast carcinoma have reported a basal-like subtype that expresses basal CK or EGFR in 9-20% of all breast cancer cases.[4-6,18] TNBC, with or without a basal CK and/or EGFR expression, has been reported to account for 16-37% of all breast cancer cases.[6,9,14] The epidemiology studies reported by Carey *et al*.[5] and Millikan *et al*.[18] used a classification that included the basal-like subtype that expressed basal CK and/or EGFR instead of the TN classification; the prevalence of TNBC, as calculated from the reported data, was 26.4% and 26.3%, respectively, for each study. Moreover, the data from these studies revealed that the basal-like subtype is a frequent finding (39% and 27.2% respectively) in premenopausal African American women. The only reported study on Korean women[19] used the immunohistochemical definition provided by Nielsen *et al*.,[4] and the authors of that study classified the breast cancers as 15.9% with the basal subtype and 23.7% with a negative subtype. Because the definition reported by Nielsen *et al*. does not include the PR status, the classification of ER-/PR+ breast cancers was different from that used by our study. There are a number of common characteristics in the TNBCs and basal-like subtypes; the TN phenotype is considered to be a simple clinical surrogate for the basal-like subtype, but they are not exactly the same.[9,20] Recent studies have demonstrated that the tumors expressing CK5/6 and/or EGFR persistently have a poorer prognosis; therefore, the use of the available five markers for the definition, including ER, PR, HER2, CK5/6 and EGFR, would likely be more useful for the predicting the prognosis than the TN definition alone.[6,16]

As previously reported, some histology diagnoses were associated with a specific immunohistochemical subtype. The typical ER-expressing tumors such as invasive lobular carcinomas, tubular carcinomas and mucinous carcinomas have been included in the luminal subtype, and most metaplastic carcinomas and medullary carcinomas have been included in the TN subtype.[21,22] A higher proportion of the HER2 subtype has been noted in microinvasive carcinomas. This is thought to be due to the fact that microinvasive carcinomas are more than 90% composed of DCIS, and the HER2 overexpression in DCIS is more frequent than that in invasive carcinomas.

The overall prevalence of DCIS co-existing with breast carcinoma was 76.1% (1,225 of 1,610 cases), and this was relatively low for the TN subtype (196 of 330 cases, 59.4%). Most of the DCIS co-existing with TNBC showed high grade histology, and a small extent of the DCIS. The p53 expression and the Ki-67 index of the DCIS correlated with that of the co-existing TNBC, as did the TN immunoprofile. In the prior studies of pure DCIS with the basal-like subtype, a significant low prevalence (6-8%) and high grade histology were also noted;[12,13] a few studies have reported that most cases with DCIS shared the same immunohistochemical features as co-existing carcinomas.[14,23] It is reasonable to assume that a precancerous DCIS and the breast carcinoma that develops from the DCIS would share similar histological and biological features; these features might be transferred from the DCIS to the invasive carcinoma. Therefore, the TN DCIS is thought to be a highly probable precursor lesion of triple-negative breast carcinoma. In addition, a prior study

reported that poorly differentiated invasive ductal carcinoma typically had little or no associated DCIS because of a rapid disease progression; these carcinomas would be included in the basal-like subtype.[12] It is thought that the low prevalence and small extent of the DCIS, with the co-existing TNBC, results from the aggressive and rapidly progressive features of the TN subtype.

There are two models that explain the evolution of invasive breast carcinoma from DCIS: "*the theory of linear progression*" and "*the theory of parallel disease*".[24] The multistep linear progression model describes that an invasive carcinoma develops from a low-grade DCIS to a high-grade DCIS by a "linear pattern", and this model is supported by many studies, including the recent report by Allred *et al*. They reported that multiple histological grades, biomarker phenotypes and intrinsic subtypes often coexist within the same DCIS, and these diverse regions probably compete for dominance, and eventually the most aggressive or poorly differentiated area prevails. This concept supports the development of well-differentiated DCIS to poorly differentiated DCIS, and this due to randomly acquired genetic defects. In the parallel model, on the other hand, low-grade invasive carcinoma tends to develop from a low-grade DCIS, and high-grade invasive carcinoma tends to develop from a high-grade DCIS; the majority of the molecular changes observed in the invasive carcinoma are evident in the DCIS. Our study identified 15 cases (12.1%) of DCIS that had a different immunophenotype from the co-existing TNBC. These discordant cases might be compatible with lesions showing the diversity of DCIS as reported by Allred; however these cases only accounted for a few cases. Most of the DCIS co-existing with TNBC expressed high grade histological features and the TN phenotype. According to the parallel model, most of the cases of invasive carcinoma with a histological high grade and the TN phenotype would be determined by the precancerous DCIS. Therefore, in the cases with TNBC, a high-grade DCIS with the TN phenotype would be considered to be a highly probable precursor lesion of the TNBC by the parallel model, but not by the linear model.

In summary, TNBCs were observed in about 20% of the total cases of breast carcinomas and they were composed mainly of invasive ductal carcinoma, NOS. Most of the medullary carcinomas and metaplastic carcinomas were also included in the TNBC. We observed a lower prevalence and a small extent of the DCIS co-existing with the TNBC, compared to the other subtypes of breast cancer. Most DCIS with TNBC exhibited a similar high grade histology with the co-existing carcinoma as well as a similar immunohistochemical TN phenotype, yet a few cases with different immunophenotypes were also noted. Therefore, the TN DCIS is a highly probable, but not obligatory, precursor lesion of TNBC.

## REFERENCES


1. Perou CM, Sorlie T, Eisen MB, *et al*. Molecular portraits of human breast tumours. Nature 2000; 406: 747-52.
2. Sorlie T, Perou CM, Tibshirani R, *et al*. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001; 98: 10869-74.
3. Sorlie T, Tibshirani R, Parker J, *et al*. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003; 100: 8418-23.
4. Nielsen TO, Hsu FD, Jensen K, *et al*. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004; 10: 5367-74.
5. Carey LA, Perou CM, Livasy CA, *et al*. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006; 295: 2492-502.
6. Cheang MC, Voduc D, Bajdik C, *et al*. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res 2008; 14: 1368-76.
7. Abd El-Rehim DM, Ball G, Pinder SE, *et al*. High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. Int J Cancer 2005; 116: 340-50.
8. Abd El-Rehim DM, Pinder SE, Paish CE, *et al*. Expression of luminal and basal cytokeratins in human breast carcinoma. J Pathol 2004; 203: 661-71.
9. Tang P, Wang J, Bourne P. Molecular classifications of breast carcinoma with similar terminology and different definitions: are they the same? Hum Pathol 2008; 39: 506-13.
10. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer 2007; 109: 25-32.
11. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncol 2007; 8: 235-44.
12. Bryan BB, Schnitt SJ, Collins LC. Ductal carcinoma in situ with basal-like phenotype: a possible precursor to invasive basal-like breast cancer. Mod Pathol 2006; 19: 617-21.
13. Livasy CA, Perou CM, Karaca G, *et al*. Identification of a basal-like subtype of breast ductal carcinoma in situ. Hum Pathol 2007; 38: 197-204.

14. Dabbs DJ, Chivukula M, Carter G, Bhargava R. Basal phenotype of ductal carcinoma in situ: recognition and immunohistologic profile. Mod Pathol 2006; 19: 1506-11.
15. Jacobs TW, Gown AM, Yaziji H, Barnes MJ, Schnitt SJ. Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. J Clin Oncol 1999; 17: 1983-7.
16. Tischkowitz M, Brunet JS, Begin LR, *et al*. Use of immunohistochemical markers can refine prognosis in triple negative breast cancer. BMC Cancer 2007; 7: 134.
17. Livasy CA, Karaca G, Nanda R, *et al*. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol 2006; 19: 264-71.
18. Millikan RC, Newman B, Tse CK, *et al*. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 2008; 109: 123-39.
19. Lee JH, Ryu WS, Park PJ, *et al*. The survival analysis of immunohistochmically defined basal and luminal subtype of breast cancer. J Korean Surg Soc 2006; 70: 7-13.
20. Reis-Filho JS, Tutt AN. Triple negative tumours: a critical review. Histopathology 2008; 52: 108-18.
21. Reis-Filho JS, Milanezi F, Steele D, *et al*. Metaplastic breast carcinomas are basal-like tumours. Histopathology 2006; 49: 10-21.
22. Bertucci F, Finetti P, Cervera N, *et al*. Gene expression profiling shows medullary breast cancer is a subgroup of basal breast cancers. Cancer Res 2006; 66: 4636-44.
23. Steinman S, Wang J, Bourne P, Yang Q, Tang P. Expression of cytokeratin markers, ER-alpha, PR, HER-2/neu, and EGFR in pure ductal carcinoma in situ (DCIS) and DCIS with co-existing invasive ductal carcinoma (IDC) of the breast. Ann Clin Lab Sci 2007; 37: 127-34.
24. Wiechmann L, Kuerer HM. The molecular journey from ductal carcinoma in situ to invasive breast cancer. Cancer 2008; 112: 2130-42.
25. Allred DC, Wu Y, Mao S, *et al*. Ductal carcinoma in situ and the emergence of diversity during breast cancer evolution. Clin Cancer Res 2008; 14: 370-8.