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ORIGINAL ARTICLE

Clinicopathologic and prognostic evaluation of invasive breast carcinoma molecular subtypes and GATA3 expression

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Summary

Purpose: *Recently, molecular subclassification of breast carcinomas has been proposed as a new prognostic parameter.*

Methods: *We classified 222 invasive breast carcinoma cases in 5 molecular subtypes by using tissue microarray (TMA) and immunohistochemistry methods. These subtypes were luminal A (estrogen receptor/ER and/or progesterone receptor/ PR positive), luminal B (ER and/or PR positive + HER2 positive), HER2-expressing type (ER and PR negative, HER2 positive), basal-like type (ER, PR and HER2 negative, positive with at least one of these myoepithelial markers: CK5/6, CK14, EGFR) and null type (ER, PR, HER2 and myoepithelial markers negative). We compared these subtypes according to their clinicopathological features and GATA3 expression.*

Results: *Luminal A was the most frequent subtype. According to overall survival rates, HER2-expressing and basal-like types had the worst prognosis, while luminal A had the best. However, luminal B had the worst prognosis according to disease free survival. Most of the squamous differentiated metaplastic carcinomas were basal-like type. Tubular and mucinous carcinomas were luminal A. Most basal-like tumors were grade III. The majority of grade I tumors were luminal A. GATA3 positivity was associated with low grade tumors and luminal A subtype.*

Conclusion: *Molecular classification can be accepted as an independent prognostic factor for invasive breast carcinomas. GATA3 expression was associated with luminal A and low histological grade. However, it wasn't shown as an independent parameter.*

Key words: basal-like type, breast carcinoma, GATA3, molecular classification

Introduction

Breast cancer is the most common malignancy in women in Turkey. Recently using DNA microarray profiling studies, biologic and clinical heterogeneity of breast carcinoma was shown and prognostically 5 distinct molecular subtypes including luminal A, luminal B, erbB2 expressing type, normal breast-like type and basal-like type were identified [1,2]. However, this system wasn't practical for use in routine studies because of high cost and fresh tissue requirement. Instead, immunohistochemistry panels were suggested to be used [3-5]. Nielsen et al. showed that an immunohistochemical panel including ER, HER2, EGFR and CK5/6 identifies basal-like tumors with 100% specificity and 76% sensitivity [3]. Kim et al. [6] used either ER or PR to identify luminal subtype and called ER and/or PR positive cases as hormone receptor (HR) positive group. Some authors used only ER, PR and HER2 for molecular classification and triple-negative cases were accepted as basal-like [7-9]. Cheang et al. [10] showed that classification using basal markers was more useful than acceptance of triple-negative cases as basal-like. SMA, CK14, CK17, C- Kit, CD10 were the other basal markers proposed to be used [4,6,11]. In several classifications, tumors with no staining for ER, PR, HER2 and basal markers were called as null type [6,10-13]. Finally, the most useful classification included luminal A (HR+ HER2-), luminal B (HR+ HER+), HER2-expressing type (HR-, HER2+), basal-like type (HR-, HER2-, and positive with at least one of basal markers) and null type [6,10,13].

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GATA3 is a luminal marker associated with ER expression [14-17]. Its expression varies according to molecular subtypes and its positivity is significantly connected with the luminal subtype [17,18]. There is no evidence about using it as an independent parameter [14,18,19]. However, it has been reported that GATA3 is associated with decreased tumor size, low tumor grade, late disease onset, HER2 negativity and long patient survival [14,19].

Methods

Patients

The study material included a series of 222 invasive breast carcinoma cases diagnosed at I.U. Cerrahpasa Medical School, Department of Pathology and treated at the Department of Medical Oncology, between 1992 and 2002. Hematoxylin & eosin (H&E) stained slides and pathology reports were reviewed. Clinicopathologic parameters including age, sex, multifocality/multicentricity (MF/MS), tumor size, histological type, histological grade, lymphovascular and perineural invasion, axillary lymph node status, and local-distant metastases were recorded. Tumor size and lymph node status were classified on the basis of TNM classification [20]. For cases with multiple tumors, the larger tumor size was used for statistics. The histological types were evaluated according to WHO-2003 classification [21]. Modified Bloom-Richardson grading system was used for histological grading [22]. Moreover, overall survival (OS) and disease free survival (DFS) for each case were recorded. ER and PR status was known for 188 cases.

Tissue microarray (TMA) construction

Representative areas of each tumor were carefully selected on H&E stained sections and marked on individual paraffin blocks. Three tissue cores (2 mm in diameter) were obtained from each selected specimen and transferred to a recipient paraffin block using a tissue-arraying instrument. Fourteen TMA blocks were constructed. Control tissue cores from nonneoplastic kidney, liver and spleen were included in each block.

Immunohistochemistry

3 μm thick sections were obtained with a microtome, transferred into positively charged slides and dried at 56°C for 12 h. Sections were deparaffinized by using xylene for 15 min and rehydrated using alcohol 100% and 96% for 15 min. Then, sections were retrieved by using EDTA 10% for EGFR, GATA3, and citrate buffer for ER, PR, HER2, CK14, CK5/6 at 750 W microwave for 15 min. After cooling for 20 min at room temperature the specimens were washed with distilled water. Endogenous peroxidase activity was blocked by incubation of slides in hydrogen peroxide solution for 10 min. After washing with phosphate buffer saline (PBS) solution for 5 min, protein blocking was performed (CAS Block, Zymed, San Francisco, USA). Then, the slides were incubated with primary antibodies for 2 h (Table 1). After washing with PBS solution they were incubated with secondary antibody (Super Picture Polymer Detection Kit, Zymed, San Francisco, USA) for 30 min. Then AEC (3-amino-9-ethyl carbazole). Substrate Solution (Zymed) was dropped and, after washing in PBS, they were stained with Mayer's Hematoxylin for 5 min.

Interpretation of immunohistochemistry

Cases were considered positive for ER, PR and GATA3 when strong nuclear staining was observed in at least 10% of tumor cells tested. HER2 immunostaining was considered positive when strong membranous staining (score 3+) was observed in at least 30% of tumor cells. Positivity for CK5/6 and CK14 was defined as the detection of at least 1 tumor cell showed strong cytoplasmic staining. Immunostaining for EGFR was interpreted as positive when at least 10% of tumor cells showed strong membranous staining (cytoplasmic staining was ignored).

Table 1. Antibodies used in the immunohistochemical study

Antibody	*Clone*	*Dilution*	*Vendors*	*Positive control*
ER	SP1	1:400	NEOMARKERS	Nonneoplastic breast
PR	NCL-L-PGR-312	1:200	NOVACASTRA	Nonneoplastic breast
HER2	COCTAIL	1:1000	NEOMARKERS	Breast carcinoma
CK5/6	D5/16B4	1:100	ZYMED	Mesothelioma
CK14	LL001	1:250	SANTA CRUZ	Skin
EGFR	F4	1:250	SANTA CRUZ	Placenta
GATA3	HG3-31	1:50	SANTA CRUZ	Nonneoplastic breast

Molecular classification

A total of 222 cases were classified as 5 molecular subtypes on the basis of immunohistochemical expressions (Figure 1). If a tumor was ER and/or PR (HR) positive but HER2 negative, it would be classified as luminal A; however, if it was either HR or HER2 positive it would be classified as luminal B. If a tumor was HR negative but HER2 positive, it would be classified as HER2 expressing type. If a tumor was triple negative but positive with at least one basal marker (CK5/6, EGFR, CK14), it would be classified as basal-like type. The tumors with no or inadequate antibody expression would be classified as null type.

Statistical analysis

Statistical analyses were performed using SPSS 10,0 software. The x^2 contingency test was used for categorical variables to determine the differences between subtypes. Survival analysis was conducted using the Kaplan-Meier method. To identify independent prognostic significance, Cox regression analysis was used. Differences at $p<0.05$ were considered statistically significant.

Results

Demographics of patients and tumor characteristics

In total, 222 invasive breast carcinoma cases were analyzed. All but 5 patients were women. Age ranged from 21 to 86 years (mean 54) for women and 53 to 92 years (mean 70) for men.

The types of surgery were modified radical mastectomy (MRM) (175 cases), breast-conserving operation (45 cases) and simple mastectomy (2 cases). Axillary dissection was performed in all but one breast-conserving operations. Tumor size varied from 1 to 10 cm. Thirty-four cases showed MF/MS and 4 cases had bilateral tumors. While 131 cases had positive lymph nodes, 87 cases were negative. Two simple mastectomy cases, a MRM case without axillary clearance and one of breast-conserving operation case were classified as "unknown lymph node status". Follow-up period was known for 197 cases and ranged from 4 to 178 months. During this period, 51 cases developed recurrence and 17 cases died of breast cancer. Time to recurrence was unknown for one case. The earliest recurrence was 3 months after diagnosis. The earliest death was 7 months from initial diagnosis. The results are summarized in Table 2.

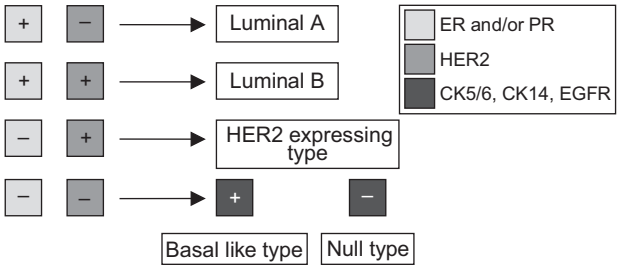

Figure 1. Molecular classification of cases on the basis of immunohistochemical expressions.

Immunohistochemical results

In this series of 222 tumors, the ratio of positive expression was 28/222 for ER, 19/222 for PR, 131/222 for both of them and 46/222 for HER2. The cases were initially subdivided into groups according to the status of the ER, PR and HER2 expression as follows: luminal A, 156 cases (70.3%), luminal B 22 cases (9.9%), HER2 expressing type 24 cases (10.8%). Eighteen of 20 triple-negative cases (8.1%) which showed positivity for one or more basal markers (CK5/6, CK14 and EGFR) were categorized as basal-like type. Two cases (0.9%) were classified as null type.

Expression of basal markers in luminal and basal-like types

While 13 (72.2%) of 18 cases showed positive reaction for CK5/6, 9 (50%) cases showed positive staining for CK14. EGFR positivity was observed in 11 (68.8%) of 16 basal-like carcinomas. CK5/6 was expressed in 5 (4.2%) of 120 luminal A and 2 (10.5%) of 19 luminal B cases. CK14 was expressed in 31 (22.3%) of 139 luminal A and 4 (19%) of 21 luminal B cases. EGFR was expressed in 41 (28.5%) of 144 luminal A and 10 (45.5%) of 22 luminal B cases (Figures 2,3). The decrease of the number of cases in each group was caused by unsuccessful staining.

Correlation of subtypes with clinicopathologic parameters

Luminal A was the most frequent subtype in both sexes (152 of 217 female and 4 of 5 male). There was no association between age and molecular subtypes. Although HER2-expressing type was the most frequent subtype that was associated with MF/MS, this result wasn't statistically significant. Tumors of ≥ 5 cm in diameter were more frequent in basal-like type than in luminal A, but without statistical significance. Most of the squamous differentiated metaplastic carcinomas

Table 2. Clinicopathologic demographics of the patients

Demographics	*Patients n*	*%*
Age (years)		
≥35	11	5
36-50	81	36.5
51-65	88	39.6
>65	42	18.9
Sex		
Female	217	97.7
Male	5	2.3
Type of surgery		
Breast conserving operation	45	20.3
Modified radical mastectomy	175	78.8
Simple mastectomy	2	0.9
Laterality		
Right	103	46.4
Left	115	51.8
Bilateral	4	1.8
Multifocality/multicentricity		
Present	34	15.3
Absent	188	84.7
Tumor size (cm)		
≤2	84	37.8
2-5	111	50.0
>5	27	12.2
Histologic subtype		
IDC, NOS	178	80.2
ILC	18	8.1
Mixed ductal and lobular carcinoma	9	4.1
Mucinous carcinoma	5	2.3
Tubular carcinoma	1	0.5
Cribriform carcinoma	1	0.5
Medullary carcinoma	1	0.5
Invasive papillary carcinoma	1	0.5
Invasive micropapillary carcinoma	1	0.5
Metaplastic carcinoma		
chondroid	1	0.5
squamous	5	2.3
Invasive apocrine carcinoma	1	0.5
Histologic grade		
I	21	9.5
II	138	62.2
III	63	28.3
Lymphovascular invasion		
Present	157	70.7
Absent	65	29.3
Number of metastatic nodes		
0	87	39.2
1-3	67	30.2
4-9	42	18.9
≥10	22	9.9
Unknown	4	1.8
Perineural invasion		
Present	58	26.1
Absent	164	73.9
Local recurrence or distant metastasis		
Present	51	23
Absent	145	65.3
Unknown	26	11.7

IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma, NOS: not otherwise specified

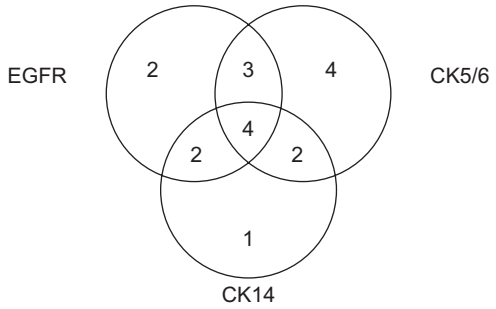

Figure 2. Expression of myoepithelial markers in basal-like type.

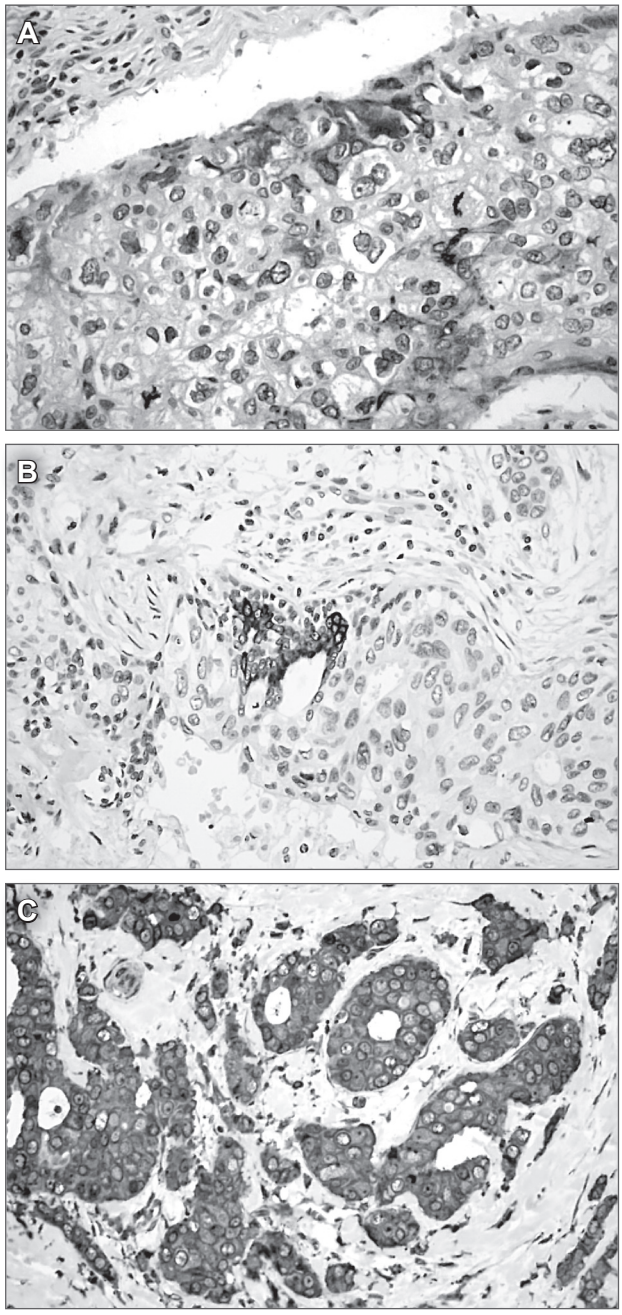

Figure 3. Basal-like type. **A:** Strong cytoplasmic immunopositivity for CK5/6 (×400). **B:** Focal cytoplasmic immunopositivity for CK14 (×400). **C:** Diffuse membranous immunopositivity for EGFR. Cytoplasmic staining was ignored (×400).

(3/5) were basal-like type. The well differentiated tumors like tubular (1/1) and mucinous (4/5) carcinomas were luminal A. There was a significant association between molecular subtypes and tumor grade (p=0.001). While most luminal A tumors were grade II, most basal-like tumors were grade III. The majority of grade I tumors (20/21) were luminal A. No association was found between molecular subtypes and axillary lymph node status, lymphovascular invasion and perineural invasion (Tables 3,4).

Table 3. Correlation of subtypes with clinicopathologic parameters

Subtypes	*Luminal A (n=156) n (%)*	*Luminal B (n=22) n (%)*	*HER2 expressing type (n=24) n (%)*	*Basal-like type (n=18) n (%)*	*Null type (n=2) n (%)*	*Total (n=222) n (%)*
Age (years)						
≤35	4 (2.5)	3 (13.7)	4 (16.7)	0	0	11 (5.0)
36-50	60 (38.5)	9 (40.9)	5 (20.8)	7 (38.9)	0	81 (36.5)
51-65	56 (35.9)	9 (40.9)	11(45.8)	10 (55.6)	2 (100.0)	88 (39.6)
>65	36 (23.1)	1(4.5)	4 (16.7)	1(5.5)	0	42 (18.9)
Sex						
Female	152	21	24	18	2	217 (97.7)
Male	4	1	0	0	0	5 (2.3)
MF/MS						
Present	22 (14.1)	5 (22.7)	6 (25.0)	1 (5.6)	0	34 (15.3)
Size (cm)						
≤2	64 (41.0)	4 (18.2)	10 (41.7)	5 (27.8)	1 (50.0)	84 (37.8)
2-5	78 (50.0)	12 (54.5)	12 (50.0)	9 (50.0)	0	111 (50.0)
>5	14 (9.0)	6 (27.3)	2 (8.3)	4 (22.2)	1 (50.0)	27 (12.2)
Grade						
I	20 (12.8)	0	0	1 (5.6)	0	21 (9.5)
II	111 (71.2)	11 (50.0)	12 (50.0)	4 (22.2)	0	138 (62.2)
III	25 (16.0)	11 (50.0)	12 (50.0)	13 (72.2)	2 (100.0)	63 (28.3)
LVI	107 (68.6)	21 (95.5)	15 (62.5)	13 (72.2)	1 (50)	157 (70.7)
PNI	48 (30.8)	5(22.7)	3 (12.5)	2 (11.1)	0	58 (26.1)
LN status	(n=154)	(n=22)	(n=23)	(n=17)	(n=2)	(n=218)
0	65 (42.2)	5 (22.7)	8 (34.8)	7 (41.2)	2 (100.0)	87 (39.9)
1-3	52 (33.8)	7 (31.8)	5 (21.7)	3 (17.6)	0	67 (30.7)
4-9	25 (16.2)	7 (31.8)	6 (26.1)	4 (23.6)	0	42 (19.3)
≥10	12 (7.8)	3 (13.7)	4 (17.4)	3 (17.6)	0	22 (10.1)

MF/MS: multifocality/multicentricity, LVI: lymphovascular invasion, PNI: perineural invasion, LN: lymph node

Table 4. Comparison of histological and molecular subtypes

Histological subtypes	*Luminal A (n=156)*	*Luminal B (n=22)*	*HER2 expressing type (n=24)*	*Basal-like type (n=18)*	*Null type (n=2)*
IDC, NOS	124	19	20	13	2
Invasive lobular carcinoma	16	0	2	0	0
Mixed ductal and lobular carcinoma	7	1	0	1	0
Mucinous carcinoma	4	0	0	1	0
Tubular carcinoma	1	0	0	0	0
Cribriform carcinoma	1	0	0	0	0
Medullary carcinoma	0	0	1	0	0
Invasive papillary carcinoma	1	0	0	0	0
Invasive micropapillary carcinoma	1	0	0	0	0
Metaplastic carcinoma					
chondroid	1	0	0	0	0
squamous	0	1	1	3	0
Invasive apocrine carcinoma	0	1	0	0	0

Correlation of clinicopathologic parameters and subtypes with survival

After excluding 25 cases with no follow up and 2 null type carcinomas, 195 cases were statistically evaluated in terms of OS. DFS was statistically evaluated in 194 cases after excluding 25 cases with no follow up and a case with unknown recurrence time. Null type carcinomas had 38 and 50 months follow up and both patients were alive with no recurrence.

Presence of MF/MS was a statistically significant factor associated with short OS (p=0.001) and DFS (p=0.0265). MF/MS was an independent factor in determining the OS (p=0.002). Tumor size was a significant parameter for DFS (p=0.0163) but not for OS. Especially, tumors that were ≥ 5 cm in diameter had a lower DFS rate. Lymphovascular invasion was a significant parameter for DFS (p=0.0344) but not for OS. The presence of metastatic lymph nodes was an independent prognostic factor (OS p=0.043 and DFS p=0.008) and statistically associated with short OS (p=0.0044). The cases with ≥ 4 lymph node metastases had shorter DFS (p=0.0006). No impact of tumor grade and perineural invasion on survival was proven.

Molecular subtype was statistically significant prognostic factor on Kaplan-Meier analysis (for OS, p= 0.0035; for DFS, p=0.0026). In addition, multivariate analysis showed that molecular classification could be accepted as an independent prognostic parameter (for OS, p= 0.007; for DFS, p=0.036). According to OS rates, HER2-expressing and basal-like types had the worst prognosis, while luminal A had the best. However, luminal B had the worst prognosis according to DFS. Luminal B had 2-fold greater risk of local recurrence and/or distant metastases than luminal A. HER2-expressing type had a mortality risk 5-fold greater than that of luminal A (Table 5; Figures 4 and 5).

GATA3 expression

GATA3 positivity was observed in 59 (27.4%) of 215 cases that were successfully stained among the total 222 cases (Figure 6). It was only associated with tu-

Table 5. Overall survival rates in molecular subtypes

Molecular subtype	*Mean overall survival (months)*	*Mean disease-free survival (months)*
Luminal A	134	112
Luminal B	95	57
HER2-expressing type	86	75
Basal-like type	84	78

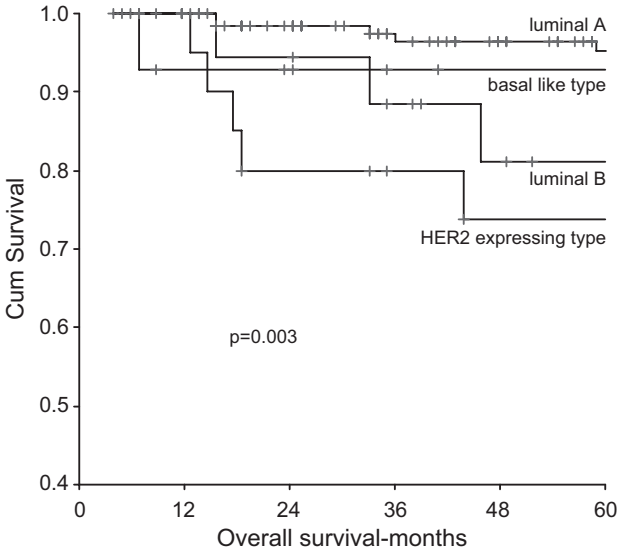

Figure 4. Overall survival of patients in breast carcinoma molecular types.

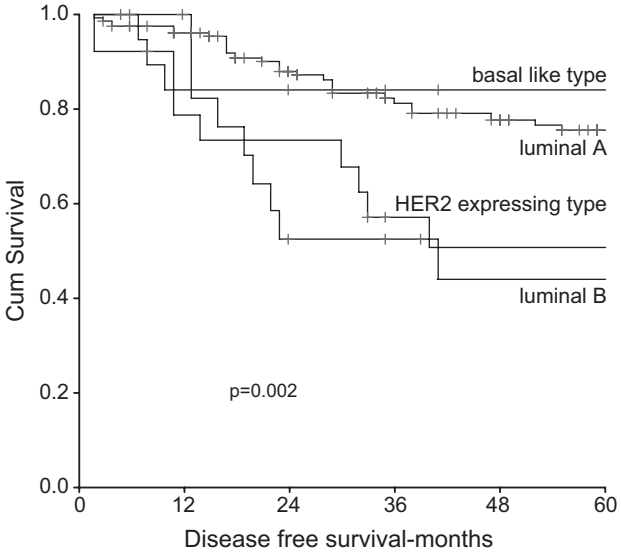

Figure 5. Disease free survival of patients in breast carcinoma molecular types.

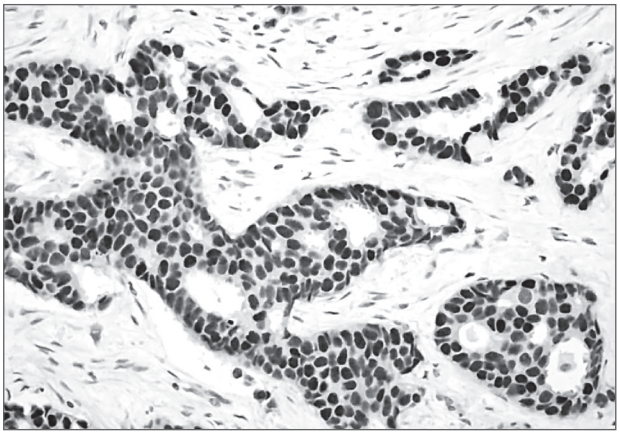

Figure 6. Strong nuclear immunohistochemical reaction for GATA3 (×400).

mor grade (p=0.016). Forty percent of grade I tumors showed GATA3 positivity. Expression of antibody was incrementally decreasing in high grade tumors (Table 6). GATA3 positivity was also statistically associated with molecular subtypes (p=0.001). The highest expression was observed in luminal A (Table 7).

Discussion

Recently, DNA microarray analyses have led to the classification of breast carcinomas into distinct molecular subgroups [1,2]. After understanding their prognostic significance, cheaper and easier classification methods were searched for use in routine studies. DNA microarray and immunohistochemistry methods were simultaneously studied and the most useful antibodies were researched [3-5]. Although there is no consensus about naming the groups and markers to define them, the most common usage includes luminal A, luminal B, HER2-expressing type, basal-like type and null type [6,10,13].

In this study, we separated 222 invasive breast carcinoma cases into five distinct subgroups by using TMA and immunohistochemistry methods as stated above. We used CK5/6, CK14 and EGFR to define basal-like carcinomas. Most cases (70.3%) were luminal A, in concordance with the literature.

In this study 18 basal-like carcinomas were compared according to staining for myoepithelial markers and 72.2% for CK5/6, 68.8% for EGFR and 50% for CK14 positivity was observed. In addition, EGFR was positive in 28.5% of luminal A and 45.5% of luminal B cases; CK14 was positive in 22.3% of luminal A and 19% of luminal B cases. In contrast, only 4.2% of luminal A and 10.5% of luminal B cases showed positive immunostaining for CK5/6. So we considered that CK5/6 was more useful to define basal-like carcinoma than EGFR and CK14.

Two cases were null type. In this study, as a limitation of TMA, immunohistochemical staining was performed only in millimetric areas of the tumors. Positive areas could be missed during sampling. This risk increases for some antibodies which are accepted positive with the detection of at least 1 tumor cell, like CK5/6 and CK14. As a result, our null type cases could be basal-like carcinomas. For this reason, using more than one basal marker seems more confident for classification.

Ge et al. [23] classified 42 male breast carcinoma cases and reported that most of them (83%) were luminal A and the rest were luminal B. In our study, similarly, 4 of 5 male cases were luminal A, while one case was luminal B.

Some authors reported that patients with basal-like carcinomas were younger than those in the luminal group [7,9,10]. In contrast, Lin et al. [24] observed that luminal A cases were younger in an Asian study because of ethnic differences. Ge et al. [23] showed that luminal B cases were younger than luminal A in men, but this finding wasn't statistically significant. We couldn't find any association between age and subtypes.

Although presence of multiple tumors wasn't an independent prognostic parameter, risk of lymph node metastasis increased in the cases with MF/MS [25]. In our study, MF/MS was an independent factor in de-

Table 6. Association of GATA3 expression and tumor grade

GATA3	*Grade I*	*Grade II*	*Grade III*	*Total*
Positive				
n	8	42	9	59
%	40.0	31.8	14.3	27.4
Negative				
n	12	90	54	156
%	60.0	68.2	85.7	72.6
Total				
n	20	132	63	215
%	100.0	100.0	100.0	100.0

Table 7. Correlation of GATA3 expression and molecular subtypes

GATA3	*Luminal A*	*Luminal B*	*HER2 expressing type*	*Basal-like type*	*Null type*	*Total*
Positive						
n	54	2	1	2	0	59
%	36.0	9.1	4.3	11.1	0.0	27.4
Negative						
n	96	20	22	16	2	156
%	64.0	90.9	95.7	88.9	100.0	72.6
Total						
n	150	22	23	18	2	215
%	100.0	100.0	100.0	100.0	100.0	100.0

termining the OS and associated with short survival. Wiechmann et al. [8] found that HER2-expressing type tumors were 1.6 times more likely to had multifocal disease compared with patients with luminal A. In our study, although HER2-expressing type was the most frequent subtype associated with MF/MS, this result wasn't statistically significant.

Tumor size is the most important prognostic parameter after lymph node status. HER2-expressing and basal-like carcinomas were reported as tumors with larger size [6-9]. In this study, it was a significant parameter for DFS but not for OS. Especially, tumors ≥ 5 cm in diameter had a lower DFS rate. Tumors ≥ 5 cm in diameter were more frequent in basal-like type than in luminal A, but this finding wasn't statistically significant.

An association between histological and molecular types was reported in the literature. Especially medullary and metaplastic carcinomas seemed associated with basal-like type [6,7,11]. None of the well differentiated tumors like tubular and mucinous carcinomas were basal-like type [6]. Discordantly with the literature, one medullary carcinoma case was HER2-expressing type in our study. The tubular (1/1) and mucinous (4/5) carcinomas were luminal A and 3/6 metaplastic carcinomas were basal-like type as in the literature.

Basal-like tumors were associated with high histological grade in many publications [6,8,9,11,26,27]. As in our study, most (72.2%) basal-like tumors were grade III and most (71.2%) luminal A tumors were grade II. The vast majority of grade I tumors (20/21) were luminal A.

Lymphovascular invasion is a minor prognostic parameter and associated with increased axillary lymph node metastasis [28]. Wiechmann et al. [8] reported that the most frequent lymphovascular invasion was seen in HER2-expressing type. We found that it was a significant parameter for DFS but not for OS. Although luminal B had the most frequent lymphovascular invasion, this result wasn't statistically significant. There was no significant association between perineural invasion and subtypes, either.

An association between lymph node status and molecular subtypes was found in many studies. HER2-expressing type was reported as the group with the most frequent axillary metastasis [6,8-10]. Kim et al. [6] reported that lymph node metastasis was more frequent in HER2-expressing type than in basal-like carcinomas, while stage was similar in both of them. Some authors suggested that the luminal type carcinomas presented with lesser nodal metastasis than basal-like type [9]. We showed that the presence of metastatic lymph nodes was an independent negative prognostic factor in breast carcinomas. However, no association was found between axillary nodal status and molecular subtypes.

We support that molecular classification could be accepted as an independent prognostic factor for invasive breast carcinomas. According to OS rates HER2 expressing and basal-like types had the worst prognosis, while luminal A had the best as in the literature [7,9,27]. HER2-expressing type had 5-fold greater mortality risk than luminal A. However, luminal B had the worst prognosis according to DFS. Luminal B had 2-fold greater risk of local recurrence and/or distant metastases than luminal A. Although both groups showed HR expression, we thought that HER2 positivity in luminal B could lead to this result. In the literature, the most frequent distant metastatic rate was observed in HER2-expressing type [6]. However, in our study DFS was longer in HER2-expressing type than in luminal B.

GATA3 was known as a luminal marker associated with ER expression [14-17]. Although its expression was associated with favorable prognosis and luminal A subtype, there has been no evidence about using it as an independent parameter so far [14,17-19]. In our study, GATA3 expression showed statistically significant frequency in luminal A and it was associated with low tumor grade. We were not able to show an impact of GATA3 expression on survival.

Finally, molecular classification looks like an important parameter to determine the clinical outcome but larger series must be tested before using it in routine practice. GATA3 expression was associated with luminal A and low histological grade, but it wasn't shown as an independent factor.

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