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ORIGINAL PAPER

Basal Phenotype in Breast Carcinoma Occurring in Women Aged 35 or Younger

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Abstract Breast cancer in the young is considered a special clinical presentation of the disease. Sixty-nine breast cancer cases diagnosed at or before the age of 35 were analyzed for common morphological and immunophenotypical features of basal-like carcinomas. Sixteen carcinomas displayed the immunophenotypical characteristics (estrogen receptor and HER2 negativity and positivity for at least one of the following basal markers: cytokeratin 5 or 14, epidermal growth factor receptor, p63) of basal-like carcinomas, and most of them demonstrated characteristic histological features (pushing borders, lymphocytic peritumoral infiltrate, central hypocellular zone or necrosis, high mitotic rate) too. These tumors were more likely to be high-molecular-weight cytokeratin: 34betaE12 and p53 positive by immunohistochemistry. The presence of a basal-like phenotype can be important as concerns systemic treatment issues and could theoretically be associated with a higher rate of BRCA1 mutations in the young, because of the overlap of BRCA1 mutation associated breast carcinomas and the basal-like phenotype.

Keywords Basal-like phenotype · BRCA1 mutation · Breast cancer in young women

Introduction

The prognosis of young women with breast cancer is controversial. The definition of young age is variable, some studies use a cut off of 35 years [1, 2], others preferred 40 years [3, 4]. There have been several reports indicating a less favorable outcome for this clinical presentation of the disease [1], and young age was found to be a factor associated with worse outcome [5]. This prognostic disadvantage may stem from the diagnosis of breast cancer being more difficult in younger women due to the limited value of mammography in this age group, but it is also likely that the disease itself is more aggressive.

As BRCA1 mutation carriers may develop breast cancer earlier than those who do not have such a genetic predisposition [3, 6], young women diagnosed with breast cancer may also have an elevated probability to carry a BRCA1 mutation [7–9]. Recent studies on breast cancer indicate that BRCA1-related carcinomas are more likely to express a basal epithelial phenotype [10–14]. Conventional histopathologic studies have shown that these tumors are often of high grade, have a high mitotic rate, areas of central necrosis or hypocellular zone, a pushing border and peritumoral lymphocytic infiltrate [15, 16]. These so-called basal-like epithelial tumors were recognized as a separate entity on the basis of gene expression profiling studies [17, 18]. Their diagnostic approach with immunohistochemistry (IHC) was based on the expression of neither estrogen receptors (ER) nor the HER2 protein, but the presence of markers characteristic of the basal epithelial cells of the normal mammary gland [19]. Nielsen and coworkers defined these tumors as ER and HER2 negative and cytokeratin (CK) 5/6 or epidermal growth factor receptor (EGFR) positive. Basal-like breast cancers are also commonly described as triple negative, i.e. negative for ER, progesteron receptor (PR) and HER2 [20, 21]. Other basal epithelial markers (e.g. p63 or CK14) can probably be added to the above IHC definition.

The incidence of basal epithelial marker positivity and of the basal-like phenotype was investigated in invasive breast carcinomas arising in patients 35 years old or younger.

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Material and Methods

The files of the Department of Pathology of the Bács-Kiskun County Teaching Hospital were searched for cases of breast cancer diagnosed in individuals aged 35 years or less between 01 January1980 and 31 December 2007.

Histological slides and pathology reports were retrieved. Most cases (n=55) were studied in tissue microarrays prepared with a tissue microarray builder kit (Histopathology Ltd, Pécs, Hungary) with two 1-mm-diameter cores per tumor. Sections from formalin fixed, paraffin embedded tissues were stained immunhistochemically for ER, PR, HER2, CK5, EGFR, CK14 and p63. Immunohistochemistry for high-molecular-weight (HMW) CK (34betaE12) and p53 was also carried out. The list of antibodies used and their dilutions are listed in Table 1.

The presence or absence of the morphological features mentioned in the "Introduction" (i.e. high mitotic rate, areas of central necrosis or hypocellular zone, a pushing border and peritumoral lymphocytic infiltrate) were looked for. A high mitotic rate was defined as a rate scoring 3 in the histological grading of breast carcinomas [22]. Then the samples were grouped into three categories separating the tumors with 3–4, 2, or 0–1 of these presumably characteristic features. The classical prognostic factors such as the age of the patients, the histological type [23] and grade [22] of the tumors and their lymph node involvement were also assessed.

Basal-like breast cancers were identified on the basis of their immunophenotype according to the definition used by Nielsen and coworkers [19] slightly modified: Negative for ER and HER2, and positive for CK5 and/or EGFR (and/or CK14 and/or p63).

For the interpretation of immunohistochemical reactions, any positivity at an adequate place (i.e. cytoplasmatic for CKs; nuclear for ER, PR, p63 and p53; and membranous for EGFR) was considered as positive, apart from HER2. This latter was interpreted according to the Herceptest evaluation scheme,[24] and 3+ staining intensity was considered as a positive finding; cases scoring 2+ were further tested by chromogenic in situ hybridization using the Zymed kit (Zymed, Invitrogen, Carlsbad, CA, USA), and were considered positive only if gene amplification was demonstrated by this means.

Statistical comparisons between different groups were made with the help of the VassarStats statistical package (R Lowry, VassarStats, Poughkeepsie, NY, USA).

Table 1 Details of the antibodies used in this study

Antibody	Manufacturer	Further identification	Dilutions used
ER	Novocastra	6F11	1:40
PR	DAKOCytomation	PgR636	Ready to use (1:2)
HER2	Novocastra	CB-11	1:80
CK5	Labvision	XM26	1:25
CK14	Novocastra	LL002	Ready to use
EGFR	DAKOCytomation	EGFR pharm Dx 2–18C9	Ready to use
p63	DAKOCytomation	4A4	1:100
HMWCK	DAKOCytomation	34betaE12	1:100
p53	DAKOCytomation	D0–7	Ready to use

CK Cytokeratin, *EGFR* epidermal growth factor receptor (HER1), *HMWCK* high-molecular-weight cytokeratin, *ER* estrogen receptor, *PR* progesterone receptor

Table 2 Details on pT and pN categories of the studied tumors

		Nodal status			
		N0	N+	Nx	Total
Tumor size	T1	9 (2)	12 (1)	2	23 (3)
	T2	8 (4)	15 (2)	4 (2)	27 (8)
	T3	–	1 (1)	–	1 (1)
	T4	–	1	1	2
	Tx	6 (2)	6 (1)	4 (1)	16 (4)
	Total	23 (8)	35 (5)	11 (3)	69 (16)

Numbers in parenthesis refer to tumors with a basal-like immunophenotype.
T1–Tx T categories of the TNM classification of breast carcinomas, *N0* node-negative status, *N+* node-positive status, *Nx* unknown nodal status

Results

Sixty-nine breast cancers were identified in young women during the studied period. The median age of the patients was 33 (mean ± SD: 31.8±3.1; range, 19–35 years).

Sixty tumors were of no special type ductal carcinomas and nine were of special types: three medullary, one metaplastic, one pleomorphic lobular, one mixed lobular, one tubular, one tubular mixed, and one mucinous mixed.

Most of the cancers (43 cases) were grade III, and only four were grade I carcinomas. Details on size and nodal status according to the TNM categories are given in Table 2. The majority of carcinomas with a basal-like phenotype belonged to the T2 category, and the proportion of node-negative cases was somewhat higher than that of non-basal-like carcinomas (8/16 versus 15/53) but this difference did not prove to be significant (p=0.1; Fisher exact test).

Sixteen tumors matched the immunophenotype of basal-like carcinomas (Table 3); all were negative for PR too, in keeping with the "triple negativity" (ER, PR and HER2 negative status) of these tumors. Eight (i.e. half) of them had three or four of the previously mentioned light microscopic morphological characteristics that were looked

Table 3 IHC staining profile of ER negative and HER2 negative and basal marker (CK5 or CK 14 or EGFR or p63) positive breast cancers

Basal markers			
CK5+ (11)	CK14+ (7)	EGFR+ (2)	p63+ (1)
			p63− (1)
		EGFR− (5)	p63+ (1)
			p63− (4)
	CK14− (4)	EGFR+ (2)	p63+ (2)
			p63− (−)
		EGFR− (2)	p63+ (−)
			p63− (2)
CK5− (5)	CK14+ (1)	EGFR+ (1)	p63+ (1)
			p63− (−)
		EGFR− (−)	p63+ (−)
			p63− (−)
	CK14− (4)	EGFR+ (4)	p63+ (−)
			p63− (4)
		EGFR− (−)	p63+ (−)
			p63− (−)

for in contrast to a single non-basal-like carcinoma. The quantitative relationship between these histological features and the basal-like immunophenotype as well as the results of p53 immunohistochemistry is demonstrated in Table 4. The proportion of p53 positive tumors seemed also higher in the carcinomas with a basal-like immunophenotype (9/16 vs 23/53), but this difference did not prove to be significant (p=0.27; Fisher exact test).

Basal-like breast cancers were defined by ER and HER2 negativity by immunohistochemistry and the positivity of basal markers (CK5, CK14, EGFR or p63). The distribution of immunostaining with these markers in the remaining tumors identified in this population of young women is shown in Table 5. Basal marker positivity was only rarely seen in ER and/or HER2 positive carcinomas (4/40) and was restricted to EGFR positivity (one case) or p63 positivity (three cases) in this series. There were 13 tumors which were negative for ER, HER2 and basal markers; seven of them were also negative for PR (triple negative, but non-basal-like carcinomas).

Table 5 Distribution and immunophenotypic characteristics of the non-basal-like tumors

HER2+ (10)	ER+ (5)	Basal marker+ (1)[a]
		Basal marker− (4)
	ER− (5)	Basal marker+ (1)[b]
		Basal marker− (4)
HER2− (43)	ER+ (30)	Basal marker+ (2)[a]
		Basal marker− (28)
	ER− (13)	–
		Basal marker− (13)

ER Estrogen receptors; basal marker: CK5, CK14, EGFR or p63
[a] p63 positive cells
[b] EGFR positivity

The relationship between immunostaining with HMWCK and the other antibodies used in this series is presented in Table 6. HMWCK positivity was not restricted to basal-like carcinomas as shown in this latter table, but most cases stained with this antibody.

Discussion

It has been proposed that basal-like breast carcinomas have an unfavorable prognosis [17, 18] although not all reports support this [25]. Medullary carcinomas are rare and their prognosis was reported to be better than that of other high grade tumors [26]. Medullary carcinomas have been reported to express CK5 in 25% of the cases [27], whereas a multiinstitutional study has found most of the typical medullary carcinomas to express a basal phenotype [28]. This discrepancy in findings may also be explained by difficulties in reproducibly diagnosing medullary cancers. Because of the difficulty and low reproducibility in diagnosing this special type of breast carcinoma, it is no longer included in the United Kingdom national breast screening reporting guidelines [23]. The three medullary carcinomas in our series also displayed the basal phenotype.

Metaplastic carcinomas of the breast comprise a heterogeneous group of malignant tumors, which also share the

Table 4 Relationship between histological features, p53 staining and basal phenotype

	Light microscopic histological features[a]				
	3 to 4 features present	2 features present	0 to 1 feature present	Total	
Basal-like immunophenotype	8	3	5	16 (23%)	p53+ (n=9)
					p53− (n=7)
Non-basal-like immunophenotype	1	11	41	53 (77%)	p53+ (n=23)
					p53− (n=30)
Total	9	14	46	69	

[a] Presence of the following features: high mitotic rate, areas of central necrosis or hypocellular zone, a pushing border and peritumoral lymphocytic infiltrate

Table 6 Relationship of HMWCK staining with immunostaining with the other markers used in this study

	ER		HER2		EGFR		CK5		CK14		p63		Basal-like phenotype (ER-, HER2– and positivity for any of the 4 other markers)	
	+	–	+	–	+	–	+	–	+	–	+	–	+	–
HMWCK+	1	19	5	15	7	13	11	9	7	13	4	16	13	7
HMWCK-	34	15	5	44	3	46	0	49	1	48	4	45	3	46

CK Cytokeratin, *EGFR* epidermal growth factor receptor (HER1), *ER* estrogen receptor, *HMWCK* high-molecular-weight cytokeratin, *IHC* immunohistochemistry, *PR* progesteron receptor, *TNM* tumor, node, metastasis

basal phenotype [29], and the single case belonging to this histological type displayed the basal IHC staining profile.

Although not a homogeneous group of tumors, both metaplastic and medullary carcinomas have distinguishing morphological features, and it seems that some of the high grade ductal carcinomas having a basal-like phenotype can be characterized by the combination of pushing borders with lymphocytic infiltrate, central necrosis or acellularity and a very high mitotic index. These morphologic features predicted the basal-like IHC phenotype in eight of the 16 tumors in the present series. Morphology seems a rather specific although not a perfect tool (specificity, 98%; sensitivity, 50%) in predicting the basal-like IHC staining profile. These data are in keeping with the results of Fulford et al., demonstrating a relationship between morphology and the basal-like phenotype [16]. Carcinomas with these morphologic changes may be more prone to give brain and pulmonary metastases, and less likely to metastasize to regional lymph nodes [30].

These data suggest that basal-like carcinomas of the breast defined by the IHC staining profile used in this and several other studies do not form a homogeneous group of tumors; not all of them may have the same poor survival. Unfortunately survival data are not available for this retrospective study.

Of the positive basal cell markers tested CK5 was the one that was the most commonly expressed by the tumor cells. Because medullary carcinomas, a subset of the basal-like carcinomas have been reported to be associated with p53 staining, this antibody was also investigated in the present series. It was found to be more often positive with the basal-like IHC phenotype, but was neither specific (57%) nor sensitive (56%) in predicting this phenotype (Table 3). The HMWCK antibody reacts with several high molecular weight cytokeratins, including CK5 and CK14, and could therefore be a possible marker of the basal IHC profile. However, it was found to give a positive staining in some non-basal carcinomas too, resulting in a sensitivity of 81% and a specificity of 87%. Likewise, lobular neoplasia was reported to stain positively with this antibody despite reacting with any of the antibodies against the specific cytokeratin components [31]. Nevertheless, in this series, the lack of HMWCK staining had a 94% negative predictive value as concerns the basal-like phenotype.

A basal-like phenotype of the mammary carcinomas was demonstrated by IHC in 16 of the 69 young patients (23%) tested, and this is slightly greater than the reported incidence (15%) of this IHC profile in the general population [10]. One possible explanation for this difference could be the higher incidence of BRCA1 mutations among breast cancer patients of young age [7–9], since a higher proportion of the mammary carcinomas arising in BRCA1 mutation carriers show a basal-like phenotype [3, 10, 13, 32]. It may therefore be proposed that carcinomas diagnosed at a young age should be tested for the basal-like IHC phenotype, especially if they demonstrate characteristic morphologic features. This would mean testing for basal cell markers (CK5, CK14, p63 or EGFR on the basis of this study) if ER, PR and HER2 are negative. When this test is positive, BRCA1 mutation analysis could also yield higher positivity rates than in cases of unselected young patients even in the absence of family history considering the association between BRCA1 mutations and the basal-like phenotype. The demonstration of the basal-like phenotype could also have treatment implications beyond the steroid hormone and HER2 negativity [33].

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