*Originals*

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# Immunohistochemical Identification of Basal-Type Cytokeratins in Invasive Ductal Breast Carcinoma – Relation with Grade, Stage, Estrogen Receptor and HER2*

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**Gene expression analyses with cDNA microarray technology identified distinct groups of breast cancers. Tumors with no ER expression could be divided into three subgroups: "basal-like" subtype, HER2-positive subtype, and "normal breast-like". "Basal-like" subtype was characterized by high expression of keratins 5 and 17, laminin and fatty acid binding protein 7. In the present study, we analyzed the usefulness of immunohistochemistry for separation of the distinct subtypes of the breast ductal carcinomas and provided further characterization of "basal-like subtype". A consecutive series of 195 primary operable invasive breast carcinomas was immunostained for HER2, ER, PGR, CK5/6 and CK17. CK5/6 or CK17 were expressed in 72 cases (36.9%), and 41 cases (21%) presented expression of CK5/6 or CK17 without ER/PGR or HER2. ER/PGR was present in 109 cases (55.9%), but in this group there were 8 cases with HER2 overexpression and 17 cases with basal-cytokeratin positivity. Similarly, in 17 out of 72 "basal-like" tumors there was ER/PGR positivity, and also in 17 of them there was HER2 overexpression. Three of these cases belonged to all three groups, representing expression of all markers. Tumor grade differed significantly ($p<0.001$) between luminal and basal cytokeratin- or HER2-positive tumors. Differences for tumor size and lymph node status were not statistically significant. Our study showed that immunohistochemistry is useful for dividing breast cancers into separate subgroups, but further analyses for better characterization of cases presenting two or three markers should be performed.**

## Introduction

Gene expression analyses with cDNA microarray technology identified distinct groups of breast cancers [2, 9, 10, 13–16]. Tumors having no ER expression could be divided into three groups: "basal-like" subtype, HER2-positive subtype, and "normal breast-like" group. "Basal-like" subtype was characterized by high expression of keratins 5 and 17, laminin and fatty acid binding protein 7. HER2+ subtype was characterized by high expression of several genes in the ERBB2 amplicon at 17q22-24 including ERBB2 and GRB7. "Normal breast-like" group presented high expression of genes known to be expressed in adipose tissue or other nonepithelial cell types as well as strong expression of basal epithelial genes and low expression of luminal epithelial genes.

Patients with the "basal-like" and HER2-positive subtypes had shorter survival times and relapse-free survival [9–11].

The presence of "basal-like" cytokeratins in breast carcinomas was also studied immunohistochemically [1, 3, 6–8, 12], and these studies showed that "basal-like" subtype might present different biological and prognostic features. In this study, we analyzed the usefulness of immunohistochemistry for separation of the distinct subtypes of the breast ductal carcinomas and provided further characterization of the basal-like subtype.

## Material and Methods

A consecutive series of 195 cases of primary operable invasive breast carcinoma (all were primary infiltrating ductal

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breast carcinomas not otherwise specified – NOS) from patients who underwent surgery in the period between 1997 and 2001 was used. At the time of surgery, 97 patients had positive lymph nodes. Paraffin embedded sections were routinely processed. Slides for immunostaining for ER, PGR and CK17 (ER, PGR from Dako, CK17 from Novocastra) were pretreated with citrate buffer in microwave oven. HER2 expression was examined with commercially available Herceptest (Dako). CK5/6 antibody - also from Dako – was applied following autoclaving with high pH buffer. Antibodies dilutions: ER – 1:35, PGR – 1:75, CK5/6 – 1:100, CK17 – 1:40. All following procedures were done according to standard protocols with EnVision kit (Dako).

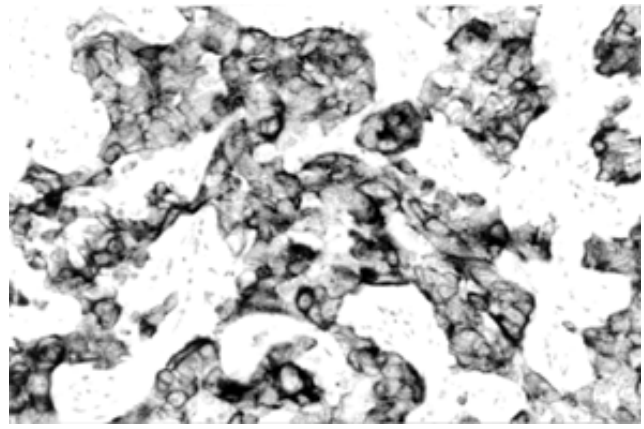

Fig. 1. Strong immunohistochemical reaction for cytokeratins CK5/6 in high-grade invasive ductal carcinoma. Magn. 400×.

## Results

CK5/6 was expressed in 68 cases, and 45 of these tumors were also CK17-positive (Figs. 1 and 2). Four tumors expressed only CK17 without CK5/6. Thus, 72 (36.9%) cases could be regarded as expressing CK5/6 or CK17.

Detailed results are presented in Figure 3 and Table 1. As presented in Figure 1, there were three distinct groups of tumors – "basal-like", HER2+ and ER/PGR+, but overlapping one another. One hundred nine (55.9%) cases were ER/PGR-positive, but in this group there were 8 cases with HER2 overexpression and 17 cases with basal phenotype. Similarly, in 17 out of 72 "basal-like" tumors there was ER/PGR positivity, and also in 17 there was HER2 overexpression. Three cases belonged to all three groups, representing expression of all

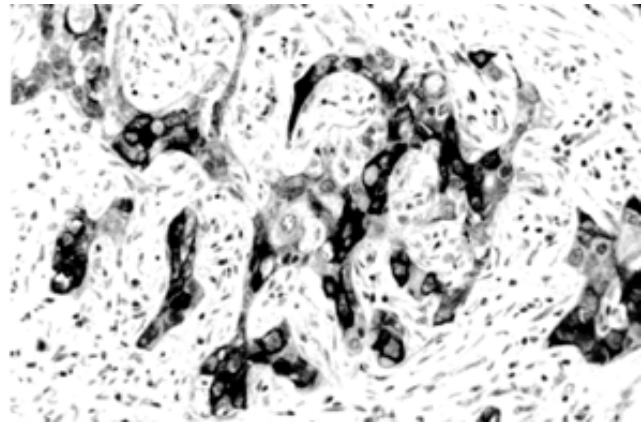

Fig. 2. Another case presenting positive reaction for cytokeratin 17. Magn. 400×.

**TABLE 1**
Staging and grading for three subtypes of breast ductal carcinoma

| | all | basal-like | luminal (ER/PGR+) | HER2 (3+) |
|---|---|---|---|---|
| T | **T1** (64–32.82%) | 21 (29.17%) | 41 (37.61%) | 8 (22.22%) |
| | **T2** (121–62.05%) | 46 (63.89%) | 63 (57.80%) | 27 (75%) |
| | **T3** (1–0.51%) | 1 (1.39%) | 0 | 0 |
| | **T4** (9–4.62%) | 4 (5.55%) | 5 (4.59%) | 1 (2.77%) |
| | **All: (195–100%)** | **72 (100%)** | **109 (100%)** | **36 (100%)** |
| N | **N(-)** (98–50.26%) | 40 (55.55%) | 52 (47.71%) | 18 (50%) |
| | **N(+)** (97–49.74%) | 32 (44.45%) | 57 (53.29%) | 18 (50%) |
| | **All: (195–100%)** | **72 (100%)** | **109 (100%)** | **36 (100%)** |
| G | **G1** (22–11.28%) | 2 (2.78%) | 22 (20.18%) | 1 (2.77%) |
| | **G2** (89–45.64%) | 27 (37.5%) | 57 (52.29%) | 14 (38.9%) |
| | **G3** (84–43.08%) | 43 (59.72%) | 30 (27.53%) | 21 (58.33%) |
| | **All: (195–100%)** | **72 (100%)** | **109 (100%)** | **36 (100%)** |

"basal-like" – CK5/6 or CK17-positive, "luminal" – ER or PGR-positive; HER2 – only 3+ positivity. These groups were overlapping each another, so the total of all three groups exceeds 195.

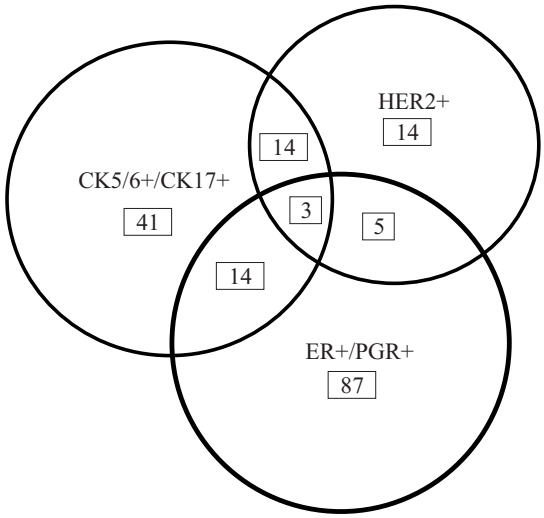


Fig. 3. Distribution of 195 cases of invasive ductal breast carcinoma between three main molecular groups: luminal (ER/PGR+), basal (CK5/6vCK17+) and HER2-positive.

markers. These tumors were T2, G2 and two of them – with nodal metastases. Thus, 41 cases (21%) presented "pure basal phenotype" with expression of CK5/6 or CK17 without ER or HER2.

Tumor grade differed significantly ($p<0.001$; Fisher's exact test) between luminal and basal cytokeratins, and between ER ("luminal") and HER2-positive tumors. Differences for tumor size and lymph node status were not statistically significant (Table 1).

## Discussion

In our study 36.9% of cases presented "basal-like" phenotype. This observation is in concordance with other studies where 16–40% of ductal carcinomas expressed basal cytokeratins – CK5/6 and/or CK17 [3, 6–8, 12, 17]. We also showed that immunohistochemistry was useful for separating infiltrating breast carcinomas into main molecular groups, but with significant overlapping between them. Biological characterization of the cases presenting two or three markers has to be examined in further studies.

This overlapping is a new observation, and has to be explained. In a study of Nielsen et al., 40% of cases were ER-positive, 20% presented HER2-positivity and 14% presented CK5/6 expression [8]. Only 13 out of 21 cases presenting basal-like phenotype on microarray analysis were CK5/6-positive with immunohistochemistry [8]. Thus, immunohistochemistry is not fully concordant with gene expression analysis. These authors found, that molecularly "basal-like" tumors are characterized by HER2 and ER negativity and positivity for HER1 (EGFR) and/or CK5/6. It means that in our study as the "basal-like" tumors should be regarded only tumors, which are ER and HER2-negative and CK5/6 or CK17-positive. We found 41 such cases (21%). Further analyses of prognostic significance for both groups are needed.

We also found, that "luminal-like" carcinomas with ER expression presented statistically significant lower grade of malignancy than other groups. Differences in tumor size and lymph node status were not statistically significant, what suggests, that potential worse prognosis is not related to differences in tumor stage.

Malzahn et al. found that majority of high-grade cancers presented expression of CK17, correlating with absence of steroid hormone receptors and short survival [7]. In 166 breast cancers studied by Korshing et al., 13 cases expressed CK5/6, and these cancers were highly proliferating and also steroid receptors-negative [6]. In a study presented by the team known for microarray analysis of breast carcinomas, 16% of cases possessed expression of CK5/6 and/or CK17, and this phenotype was associated with poor clinical outcome, independently of tumor size, tumor grade, HER2 status and ER status [12].

Abd El-Rehim et al. examined prognostic significance of the expression of "basal" cytokeratins in 1,944 cases of invasive breast cancers [1]. Approximately 30% of the cases presented basal phenotype, correlating with histological grade, tumor size, local and regional recurrence, distant metastases and death from breast cancer. Foulknes et al. obtained similar results: CK5/6 positive tumors were more likely to be ER-negative, P53-positive, cyclin E-positive, occurred more likely in younger women, and correlated with larger size and higher grade [3]. Moreover, they were also more likely to occur in *BRCA1* mutation carriers [3].

Microarray technology provided other important prognostic informations for patients with breast cancers. Van't Veer et al. selected a group of genes, which expression strongly correlated with survival in breast cancer patients [14, 15]. The same team had proved, that this set of 70 genes provided a powerful independent prognostic tool for predicting outcome for patients younger than 53 years without lymph node metastases, and was more effective than standard prognostic systems based on clinical or histological criteria (St Gallen, WHO consensus) [13]. Unfortunately, in other analyses – although also presenting important prognostic relationship and predicting values – different sets of genes were proposed, thus much more further studies are needed [2, 4, 5, 12, 16].

Our study showed that immunohistochemistry was useful for dividing breast cancers into separate subgroups, but further analyses for better characterization of cases presenting "pure" phenotype and those presenting two or three markers should be performed.

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