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Expression of CK5 basal cytokeratin in primary breast carcinoma

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Abstract

Background: CK5 positive cells represent progenitors for glandular and myoepithelial lineages of mammary epithelium. During epithelial differentiation there is a gradual decrease of CK5 expression. In case of benign lesions the proliferating luminal cells show a high expression of CK5. Contrary, the majority of malignancies which are derived from differentiated glandular cells line do not reveal immunohistochemical staining with CK5 marker. The aim of this study was to compare the expression of basal cytokeratin CK5 vs hormone receptors, HER2, Ki67 and molecular subtype's immunohistochemically defined in the primary breast carcinomas of NST type.

Material and methods: We processed 108 invasive breast carcinomas of NST type. The specimens were formalin-fixed and paraffin-embedded as traditionally. Sections were immunostained (ER, PR, HER2, CK5 and Ki67) automatically with Leica Bond-Max autostainer.

Results: Breast carcinoma of NST type was in majority of cases CK5 negative (94 cases/87%). The positive CK5 cases had a high grade of differentiation. CK5 negative tumors were usually hormone positive, but in 8 cases/6.5% a combined simultaneous CK5-ER (PR) positive expression was determined. From 22 HER2 positive cases, 16 were CK5 negative. CK5 value correlated statistically significant with all used markers, except grade of differentiation: a positive Pearson coefficient was determined in relation to HER2 and Ki67, and a negative one compared to hormone receptors and molecular subtype.

Conclusions: We support CK5 potential value in molecular subtype's differentiation. Breast carcinoma of NST type is usually CK5 negative and hormone positive. The presence of cases with simultaneous expression of CK5 and hormone receptors is an open field to debate the existence of other, transient molecular subtypes and we expect a further confirmation in larger study groups.

Key words: molecular subtypes, invasive carcinoma of NST type, basal cytokeratin CK5.

Introduction

The secretory portion of the normal breast consists of the following five distinct cell populations: committed stem (progenitor) cells which are CK5 positive, glandular precursor cells which express all spectrum of cytokeratins ($CK5^+$/ $CK8/18/19^+$), glandular end cells, positive for luminal cytokeratins ($CK8/18/19^+$), myoepithelial precursor cells positive for $CK\ 5/6^+$ and SMA^+ (smooth muscle actin), and myoepithelial mature cells, SMA positive. The CK5 positive cells represent progenitors for both glandular and myoepithelial lineages of mammary epithelium. During epithelial differentiation there is a gradual decrease of CK5 expression. In case of benign lesions the proliferating luminal cells express in excess CK5 protein and opposite, in case of the majority of malignancies glandular cells line do not reveal immunohistochemical staining with CK5 marker [1; 2].

Breast cancer is a heterogeneous disease with different clinical outcomes. It is one of the most common cancers in females worldwide. The modern classification purposes to divide it into at least five molecular subtypes which are: two hormone positive types (Luminal A and Luminal B) and three hormone negative types (HER-2 expressing, Basal-like, and Normal breast-like), each with distinct clinical features, and different prognosis [3]. Nielsen et al. purposed to differentiate immunohistochemically these subtypes by a panel of four antibodies (ER, HER1, HER2, and cytokeratin 5), point of view sustained also by Goldhirsch et al. (2013) that supplemented this panel with Ki67 as a marker of proliferation [4; 5].

The aim of this study was to compare the expression of basal cytokeratin CK5 *vs* hormone receptors, HER2, Ki67 and molecular subtypes immunohistochemically defined in the primary breast carcinomas of NST type.

Material and methods

Patients. There were investigated primary breast carcinomas of 108 patients, 33-86 years old from the Oncological Institute, the Republic of Moldova during 2012-2013 years. No drug therapy preceded and all patients underwent radical mastectomy and lymph nodes dissection.

Tissue processing and immunohistochemistry. The specimens were fixed in 10% phosphate buffered formalin for 24-48h and paraffin (Paraplast High Melt, Leica Biosystems) embedded as usual. For histopathological assessment 4-6 μm sections were cut and stained with hematoxylin and eosin. Two independent histologists reviewed the cases. Discrepancies in diagnoses were solved by consensus with simultaneous viewing. Histological grade was scored by the Scarff-Bloom-Richardson grading system. The immunohistochemical assessment included 5 markers: for ER (clone Er/6F11), PR (clone Pr16), and human epidermal growth factor receptor 2 (HER2/polyclonal), marker of proliferation Ki67 (clone Ki67/ K2) and basal cytokeratin CK5 (clone CK5/ XM26) (tab. 1). Specimens were processed automatically on Leica Bond-Max autostainer (Leica Microsystems GmbH, Wetzlar, Germany). The hematoxylin solution, Harris modified (HHS32, Sigma-Aldrich) was used for counterstaining.

Microscopic evaluation. Ki67 marker, as well as hormone receptors were counted using a semi-quantitative method performed by Suciu et al. (2014) [6]. For Ki67 marker we used a 14% threshold as a limit to distinguish positive/negative cases [5].

The anti-ER and anti-PR markers were scored as a percentage of nuclear positive stained cells at least to 1000 cells. We followed the guidelines of ER and PR assessment purposed by Allred, which are combining the percentage of positive cells with intensity of nuclear staining [7]. The cases scored as +1 – +3 were considered positive. The threshold of positivity was 10%.

The HER2 status was interpreted in accordance with ASCO (American Society of Clinical Oncology) recommendations [8]: $HER2^{+0}$ – if no staining observed or weak, barely perceptible membrane staining until 10% of cells; $HER2^{+1}$ – in case of a weak membrane staining of >10%; $HER2^{+2}$ – in case of incomplete, weak/moderate circumferential membrane staining of more than 10% of tumor cells or complete circumferential intense staining less than 10% of cells; $HER2^{+3}$ – in case of intense, circumferential staining of more than 10% of tumor cells. Cases with HER2 scored as **+2** and **+3** were considered positive. The positive cells of normal ducts served as internal control.

The CK5 expression was interpreted as Azoulay et al. previously defined: **0** – no tumor cells stained; **+1** – less of 10% of tumor cells stained; **+2** – 10-50% of positive tumor cells; **+3** – more than 50% of tumor cells stained [9]. Expression was scored positive (>0) if any cytoplasmic and/or membranous staining tumor cells were observed.

Table 1

The surrogate markers: source, dilution, systems of detection and retrieval, time of incubation

Antibody/Clone	Source/incubation time /dilution	Detection/time	Retrieval system/time
ER/6F11	Leica Biosystem Newcastle Ltd, Newcastle Upon Tyne, UK/15 min/RTU	Bond Polymer Refine Detection System (Leica Biosystems, Newcastle Upon Tyne, UK), 15 min	Bond Epitope Retrieval Solution 1, (Leica Biosystems, Newcastle Upon Tyne, UK)/20 min
PR/16			
HER2 /polyclonal	Dako Glostrup Denmark/30 min/RTU	EnVision-HER/30 min	Dako Target Retrieval Solution, pH6/20 min
Ki67/K2	Leica Biosystem Newcastle Ltd, Newcastle Upon Tyne, UK/15 min/RTU	Bond Polymer Refine Detection System (Leica Biosystems, Newcastle Upon Tyne, UK), 15 min	Bond Epitope Retrieval Solution2, (Leica Biosystems, Newcastle Upon Tyne, UK)/20 min
CK5/ XM26			

RTU: ready to use

The results were grouped in 5 subgroups: 1. ER^+ (and/or PR^+), $HER2^-$, $CK5^-$, Ki67<14 as Luminal A; 2. ER^+ (and/or PR^+), $HER2^+$, $CK5^-$ or ER^+ (and/or PR^+), $HER2^-$, $CK5^-$, Ki67>14 as Luminal B; 3. ER^-, PR^-, $HER2^+$, $CK5^-$ as HER2 over-expressed; 4. ER^-, PR^- and $HER2^-$, $CK5^+$ as Basal-like; 5. ER^-, PR^-, $HER2^-$ and $CK5^-$ as 5NP (5 negative phenotype).

Image acquisition and data processing. Slides were examined on Nikon Eclipse 80i microscope with Nikon DS-Fi1 installed camera by using Nis-elements 2.30 imaging software (Nikon Instruments Europe BV). A MS Access 2007 database was used to store and group the data.

Statistical analysis. The WINSTAT 2012.1 (R. Fitch Software, Bad Krozingen, Germany) software was used for a descriptive statistics, the mean value, standard error of mean and median were determined for Ki67. For all the tests a $P \leq 0.05$ was considered significant. A Pearson's correlation (r) was used to determine the relationship between different variables for a $P \leq 0.05$. The strength of the correlation was appreciated as: .00-.19 – "very weak"; .20-.39 –"weak"; .40-.59 – "moderate"; .60-.79 –"strong"; .80-1.0 as "very strong".

Ethics. This study has been approved by the Ethics Committee of the "Nicolae Testemitanu" University of Medicine and Pharmacy from Chisinau, the Republic of Moldova (approval number 21/13/31.03.2014).

Results

The CK5 was evaluated as negative in 94 cases/87%. The ER marker was positive in 88 cases/81,5%, the PR in 77 cases/71,3% and HER2 in 22 cases/20,4%. The Ki67 was encountered at high level (more than 14% of positive cells) in 58 cases/53,7% with a mean of 21,46±1,94 and median 15.

The G2 grade of differentiation was the most frequent registered (60 cases/55.6%), followed by G3 (40 cases/37%) and G1 (8 cases/7.4%). In relation to CK5 expression we realized that most of CK5 negative cases showed G2 and G3 grade. We have to mention that majority of G1 cases (7 from 8) were CK5 negative too (tab. 2).

By comparing the scores of markers expression we realized that majority of hormonal positive and HER2 negative cases had a lack of CK5 expression (tab. 3).

Table 2

CK5 expression in relation to grade of differentiation

CK5 expression	Grade of differentiation	No	%	
0	G1	7	6,5	87%
0	G2	53	49,1	
0	G3	34	31,5	
1	G2	5	4,6	13%
1	G3	2	1,9	
2	G1	1	0,9	
2	G2	1	0,9	
2	G3	2	1,9	
3	G2	1	0,9	
3	G3	2	1,9	

Table 4

CK5 expression grade *vs* molecular subtype

CK5 expression	Molecular subtype	No	%	
0	5NP	4	3,7	87%
0	Her2	6	5,6	
0	Luminal A	36	33,3	
0	Luminal B	48	44,4	
1	Basal-like	1	0,9	13%
1	Her2	1	0,9	
1	Luminal A	1	0,9	
1	Luminal B	4	3,7	
2	Her2	1	0,9	
2	Luminal A	1	0,9	
2	Luminal B	3	2,8	
3	Basal-like	1	0,9	
3	Her2	1	0,9	
Total		108	100,0	

Table 3

CK5 expression grade in relation to hormone receptors and HER2 scores

CK5	ER	No	%	CK5	PR	No	%	CK5	HER2	No	%
0	0	13	12,0	0	0	24	22,2	0	0	74	68,5
0	1	4	3,7	0	1	8	7,4	0	1	4	3,7
0	2	11	10,2	0	2	14	13,0	0	2	6	5,6
0	3	66	61,1	0	3	48	44,4	0	3	10	9,3
1	0	3	2,8	1	0	3	2,8	1	0	5	4,6
1	1	1	0,9	1	1	1	0,9	1	3	2	1,9
1	3	3	2,8	1	2	1	0,9	2	0	2	1,9
2	0	2	1,9	1	3	2	1,9	2	3	2	1,9
2	3	2	1,9	2	0	2	1,9	3	0	1	0,9
3	0	2	1,9	2	2	2	1,9	3	2	2	1,9
3	1	1	0,9	3	0	2	1,9				
				3	3	1	0,9				
Total	108 cases/100%										

Table 5

Pearson's correlation between surrogate markers, molecular subtype and grade of differentiation

	CK5	ER	PR	HER2	Ki67	Subtype	Grade
CK5							
r		-0,35	-0,20	0,22	0,41	-0,27	0,07
p		**0,0001**	**0,0212**	**0,0124**	**0,0000**	**0,0023**	0,2332
Er							
r	-0,35		0,52	-0,39	-0,30	0,67	-0,08
p	**0,0001**		**0,0000**	**0,0000**	**0,0009**	**0,0000**	0,2056
Pr							
r	-0,20	0,52		-0,24	-0,17	0,47	-0,18
p	**0,0212**	**0,0000**		**0,0071**	**0,0363**	**0,0000**	**0,0360**
HER2							
r	0,22	-0,39	-0,24		0,11	-0,54	0,00
p	**0,0124**	**0,0000**	**0,0071**		0,1195	**0,0000**	0,4836
Ki67							
r	0,41	-0,30	-0,17	0,11		-0,38	0,25
p	**0,0000**	**0,0009**	**0,0363**	0,1195		**0,0000**	**0,0047**
Subtype							
r	-0,27	0,67	0,47	-0,54	-0,38		-0,15
p	**0,0023**	**0,0000**	**0,0000**	**0,0000**	**0,0000**		0,0618
Grade							
r	0,07	-0,08	-0,18	0,00	0,25	-0,15	
p	0,2332	0,2056	**0,0360**	0,4836	**0,0047**	0,0618	

Note: Grade – grade of differentiation; Subtype – molecular subtype; r – Pearson correlation coefficient. Statistical significant values are given in Bold.

The most commonly determined molecular profile in our research was Luminal B (55 cases/50,9%), followed in regression by Luminal A (38 cases/35,2%), HER2$^+$ (9 cases/8,3%), 5NP (4 cases/3,7%) and Basal-like (2 cases/1,9%). Three molecular subtypes, Luminal A, Luminal B and HER2 were determined in both groups, CK5 positive and CK5 negative, while 5NP was registered only in CK5 negative group and Basal-like in CK5 positive group only (tab. 4).

The CK5 expression correlated weakly, but statistically significantly with all markers, except grade of differentiation (tab. 5).

Discussion

Abd El Rehim et al. (2004) consider that the secretory portion of the normal breast consists of the following five distinct cell populations: committed stem (progenitor) cells which are CK5 positive, glandular precursor cells which express all spectrum of cytokeratins (CK 5$^+$/CK8/18/19$^+$), glandular end cells, positive for luminal cytokeratins (CK8/18/19$^+$), myoepithelial precursor cells positive for CK5/6$^+$ and SMA$^+$ (smooth muscle actin), and myoepithelial mature cells, SMA positive [10]. By this, CK5 positive cells in fact represent progenitors for both glandular and myoepithelial lineages of mammary epithelium. During epithelial differentiation there is a gradual decrease of CK5 expression, associated with an increase in expression of CK8/18/19 in the glandular cells, and smooth muscle actin in the myoepithelial cells along the pathways of differentiation. By the Bocker W et al. (2002) data, in the lactating breast, there is a segregation of epithelial structures into CK8/18 expressing secretory zone and the proliferative zone which harbors cells of both glandular (CK8/18$^+$) and basal /myoepithelial (CK 5/6$^+$) type [11].

In case of benign lesions the proliferating luminal tumors show a high number of CK5/6 positive cells because of proliferation of both glandular and basal cells [12]. In Heatley M et al. (1995) opinion, the majority of malignancies which are derived from differentiated glandular cells line do not reveal immunohistochemical staining with CK5/6 leading, by this explaining both CK5 negativity in most lesions of atypical hyperplasia and ductal carcinoma in situ [13].

Genes expression profiling has identified various breast carcinoma classes with prognostic significance [3; 14].The

authors purposed initially to distinct 5 subtypes, as Luminal A, Luminal B, HER2+, Normal breast–like, and Basal-like. The luminal tumors were defined as hormone receptor–positive and negative for HER2 and usually tend to have a good prognosis. In contrast, HER2+ tumors are negative for hormone receptors and positive for HER2 and have been shown to have poor prognosis. The existence of normal breast–like tumors is still debated, but majority of researches consider that it expresses genes characteristic of adipose tissue and other non-epithelial breast cells, with a relatively poor prognosis.

Among all the molecular classes, Basal-like breast carcinoma seems to have the worst prognosis [15]. These tumors are negative for hormone receptors and HER2, and positive for CK5 and/or HER1 [16]. Basal-like tumors are also the most common type of tumors in patients with germline BRCA1 mutations. Such tumors are considered as high proliferative ones, with a low cellular differentiation [17; 18]. Such results are in line with our data, in which from 14 CK5 positive cases only one was evaluated with G1. In accordance with Sood et al. (2014) the CK5 did not show statistically significant correlation with age, tumor size and stage, histological type, the state of tumor margins, presence of lymphoid infiltrate and necrosis, lymph node status, and Ki67 positivity [18]. Authors reported a single significant correlation, CK5 vs tumors' grade, result debated by Rao et al. (2013) and confirmed by us: CK5 correlated significantly with all studied markers, except grade of differentiation [17] (tab. 5).

Some authors debate in the literature whether triple-negative tumors (negative for hormone receptors and HER2, CK5 positive) are synonym with Basal-like carcinoma [19]. Cheang et al. (2008) by using additional markers identified a cohort of patients with a significantly worse outcome in the group of triple-negative tumors, it means that breast carcinoma is not homogenous even inside the molecular subtypes [20]. This is in line with present research, where positive CK5 was determined in all molecular subtypes, defined by surrogate markers in accordance with Goldhirsch et al. (2013) criteria [5] (tab. 4). Moreover, in 9 of 14 cases CK5 positive marker was associated with Luminal phenotype.

Conclusions

We support CK5 potential value in molecular subtype's differentiation. Breast carcinoma of NST type is usually CK5 negative and hormone positive. The presence of cases with simultaneous expression of CK5 and hormone receptors is an open field to debate the existence of other, transient molecular subtypes and we expect a further confirmation in larger study groups.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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