

ORIGINAL PAPER

Relevance of the immunohistochemical expression of cytokeratin 8/18 for the diagnosis and classification of breast cancer

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Abstract

Purpose: The aim of our study was to characterize and describe the different immunohistochemical expression patterns of cytokeratin 8/18 (CK8/18) in breast tumors and to make a correlation between histopathology, immunohistochemistry for CK8/18 and its possible diagnostic value of this pair of keratins for molecular classification of breast cancers. **Material and Methods:** Forty cases of breast tumors immunostained with monoclonal antibodies against CK8/18 using a polymer based detection system and diaminobenzidine as chromogen were microscopically evaluated in normal and tumor breast tissue concerning the intensity, distribution and density of positive cells. Association with histopathology and nuclear grade were also studied. **Results:** Three different models of positive reaction were found: (1) normal cytoplasmic with intense and diffuse pattern, (2) aberrant membrane pattern and (3) aberrant cytoplasmic granular pattern associated with membranous positive reaction. Normal expression of CK8/18 was found in 23 cases of breast cancer, aberrant membranous in nine cases and aberrant with granular pattern in four cases. Further studies will be needed to elucidate these differences and possible correlation with other molecular markers.

Keywords: cytokeratin 8/18, breast tumors, immunohistochemistry.

Introduction

Keratins are epithelia-specific intermediate filament proteins, which are expressed in a tissue-specific manner [1]. Around 50 keratin genes were discovered across the species. Keratins were classified according to their molecular weight and isoelectric points. They were further subdivided into two subtypes, type I, which are acidic and have low molecular weight, and type II, which are basic or neutral and have high molecular weights. Keratins are obligatory heteropolymers, and the expression of at least one member of each subfamily is essential for proper filament formation [2].

Two distinct types of epithelial cell are found in the human mammary gland: basal (and/or myoepithelial) cells and luminal epithelial cells [3, 4]. In normal mammary epithelium, luminal cells usually express CK8, 18, and 19, which are typical for simple epithelia. Most malignant breast tumors derived from simple breast epithelium, and monoclonal antibodies directed against CK18 were used to identify primary and metastatic breast cancer cells in numerous investigations. Molecular classification of breast cancer is a subject of debate for many researchers [5, 6]. In the normal female breast, an increasing degree of "maturation" indicated by the expression of CK8/18 dramatically changed the expression of various growth

factor receptors, cell cycle-associated proteins, and hormone receptors. The estrogen receptor positive tumors were characterized by the relatively high expression of many genes expressed by breast luminal cells. This connection was further corroborated using immunohistochemical analysis and antibodies against the luminal cell keratins 8/18. The percentage of EGFR, cyclin A, Mib-1, and p53 strongly positive tumors clearly decreases with increasing CK8/18 expression [6].

Our purpose was to study in this work the expression patterns of CK8/18 in breast cancer and to find if this marker would be included in the molecular classification of breast tumors as diagnostic and/or prognostic marker.

Material and Methods

Forty cases of breast tumors from patients aged between 26 and 85-years-old were included in our study. Biopsies were collected by open surgery and fixed in neutral buffered formalin for 24 hours.

Paraffin embedded specimens were sliced at 5 µm thickness and immunostained with monoclonal antibodies against CK8/18 (clone DC10) using an EnVision based polymer detection system and 3,3'-diaminobenzidine as chromogen.

Counterstain with modified Lillie's Hematoxylin

was followed by mounting in a permanent mounting media.

The specimens were microscopically evaluated in normal and tumor breast tissue concerning the intensity, distribution and density of positive cells. Association with histopathology and nuclear grade were also studied.

✚ Results

Cytokeratin 8/18 had a constant but heterogenous expression in the luminal epithelial cells of normal human mammary gland and it was not found in the basal and myoepithelial cells. Normal luminal cells were intensely stained with a diffuse cytoplasmic pattern (Figure 1, a and b).

This cytoplasmic and diffuse pattern was considered as normal expression and remained positive in the normal ductal elements included in the malignant proliferation. Atypical ductal hyperplasia associated with carcinoma showed a similar expression pattern as mentioned for normal mammary gland.

From 40 cases with infiltrating mammary tumors (most of them ductal invasive carcinomas), 36 (90%) were positive for cytokeratin 8/18, and four (10%) had no reaction for the same marker. Over 90% if not all tumor cells strongly expressed CK8/18 in positive cases. No correlation was found between histopathology, nuclear grade and immunohistochemical expression of CK8/18.

We observed three different distribution patterns of the positive reaction: (1) intensely and diffuse cytoplasmic stain similar with normal mammary gland; (2) aberrant with intensely membrane staining and lining sometimes by a positive reaction at the periphery of the cytoplasm; (3) aberrant cytoplasmic granular pattern combined with intense membrane staining. No differences in intensity of positive cells were found between the tumor positive areas. Invasion front consisted of intensely stained cells (Figure 2a). Apocrine carcinoma had a similar intensity and density of positive tumor cells (Figure 2b).

Membrane pattern was defined as the presence of positive reaction restricted to the membrane of tumor cells similar with the distribution of positive reaction for HER-2/neu oncoprotein (Figure 3a).

The thickness of positive membrane pattern had little variations between cases. The third type of expression with aberrant cytoplasmic pattern associated with membrane positive reaction was very rare and it was also found in DCIS associated with invasive carcinoma (Figure 3b).

Based on these criteria, we identified normal expression of CK8/18 in 23 cases of breast tumors, aberrant membrane type in nine cases and aberrant granular type combined with membrane reaction in four cases. Significance of expression patterns described above is still unclear, but we observed a high correlation between aberrant granular pattern and overexpression of HER-2/neu oncoprotein. Aberrant membrane pattern was frequently associated with inflammatory reaction of breast tumors stroma.

A particular expression pattern was found in mammary Paget disease. Paget cells were positive and could not be differentiate from Toker cells. The large sized tumor cells from proliferation front had a cytoplasmic heterogenous positive pattern for CK8/18, but the intensity of the staining was lower than those found in normal mammary gland and ductal invasive carcinoma.

✚ Discussion

For more than 20 years, cytokeratins were used only as a marker for identifying carcinomas. Regulatory changes in CK expression at the transcriptional and post-transcriptional level have been described in experimental studies on epithelial tumor cells, challenging the view that CKs are only marker proteins [7–9]. Mouse gene knockout studies concerning cytokeratins showed that the double deletion of CK18 and 19 produced the complete lack of a functional CK skeleton, which caused embryonic lethality [10].

Nowadays, carcinomas that arise from breast, lung, gastrointestinal and prostate epithelium represent the majority of cancers. Epithelial cells architecture is largely determined by cytoskeletal protein, which are part of an intracellular protein network comprising the cytoskeleton itself [11]. The same authors classify the expression of CK8/18 in breast tumors according to the expression in normal breast tissue, in three categories on a TMA study on 1087 cases, but they used only percent of positive cells found in breast tumors compared with normal one.

Our findings showed that almost all cases had all tumor cells positive for CK8/18. Differences between these two studies concerning the percent of positive cells probably derive from a smaller tumor area, which was assessed in the previous study compared with our study.

Immunohistochemical expression patterns of various tissue markers in breast cancer are very important for their utility as putative targets for therapy. Interpretation of HER-2/neu protein overexpression in breast cancer includes the percent of tumor positive cells correlated with intensity and distribution of membrane pattern. Only positive cases scored as +3 for HER-2/neu oncoprotein are selected for therapy with Herceptin, but not all cases respond in the same manner. Changes in gene profiling for different markers could explain such an expression and therapy response heterogeneity found in breast tumors, not only for HER-2/neu, but also for cytokeratin 8/18.

Schaller G *et al.* [12] and Becker M *et al.* [13] reported that loss of these keratins was associated with a significantly worse prognosis. In breast cancer cell-lines, invasiveness in vitro and metastatic potential in athymic mice also correlated inversely with the degree of CK18 expression.

Previous studies made by Sommers CL *et al.* [14, 15], and Thompson EW *et al.* [16] showed that breast cancer cell lines became more aggressive as keratin filaments were replaced by vimentin, the intermediate filament-protein of mesenchymal cells.

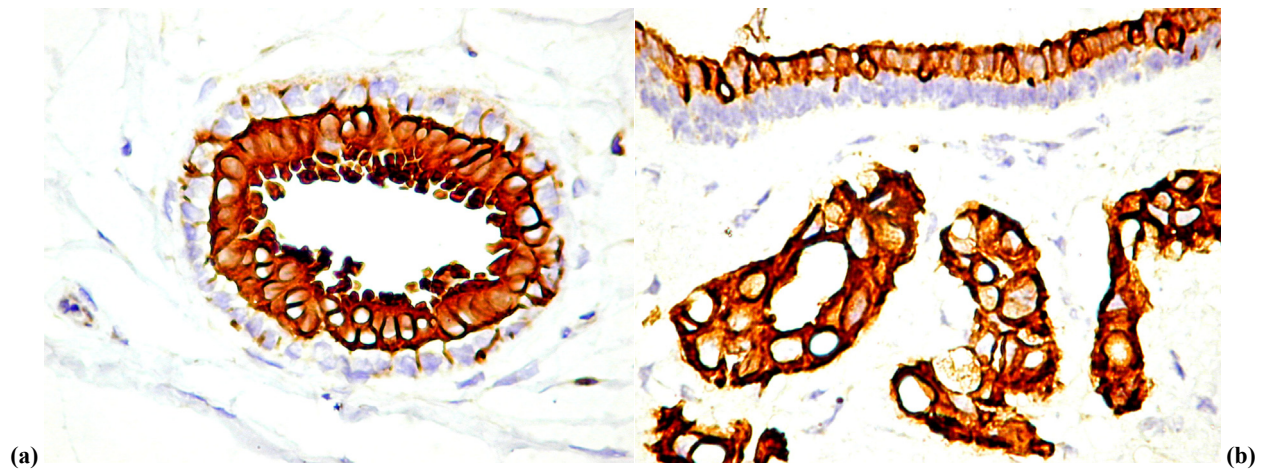


Figure 1 – Positive staining for CK8/18 of normal breast luminal cells with intense and diffuse cytoplasmic pattern considered as normal expression. Note the lack of immunostain for basal and myoepithelial cells (a, $\times 400$). Normal expression was also found in ductal epithelial cells entrapped into malignant areas (b, $\times 400$)

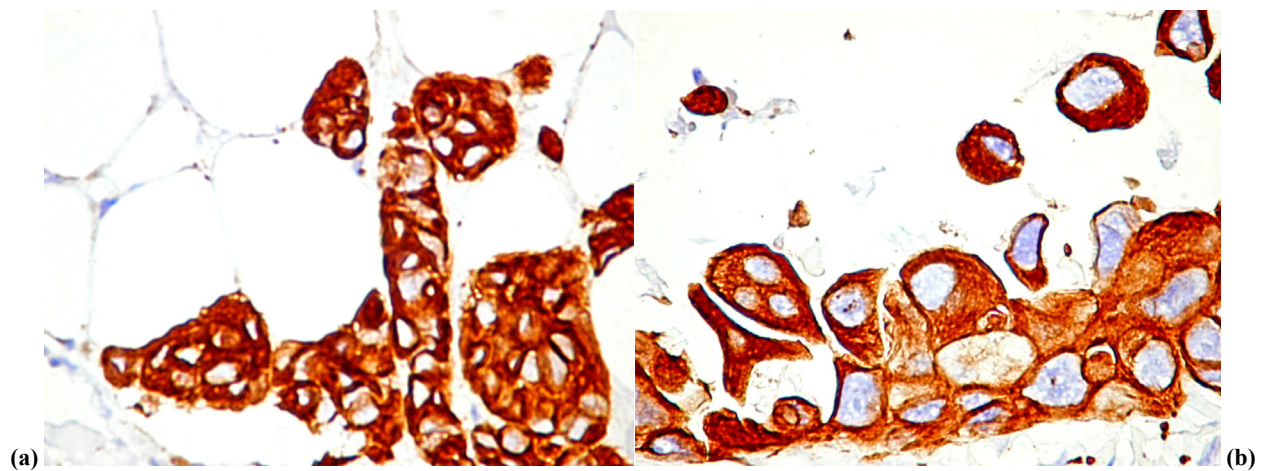


Figure 2 – Intense stain for CK8/18, homogeneous for all proliferating cells from invasion front (a, $\times 400$). The same pattern was found in special types of carcinoma like apocrine carcinoma (b, $\times 400$)

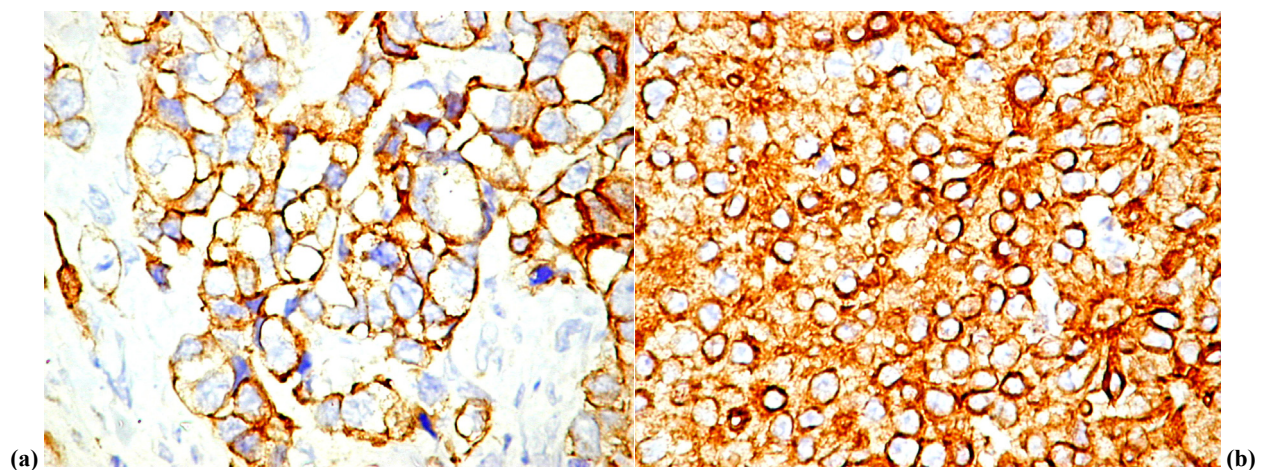


Figure 3 – Membrane positive reaction for CK8/18, with similar distribution of HER-2/neu positive staining (a, $\times 400$). Granular cytoplasmic reaction with membrane staining pattern was occasionally observed in our study (b, $\times 200$)

The breast cancer cell-lines MCF7, ZR-75-1, SK-BR-3, and MDA-MB-231 have been shown to produce large amounts of keratin 8 and keratin 18 [17]. MDA-MB-231 cells, which express virtually no CK18, are characteristically aggressive invasive

and metastatic [18, 19]. CK18 gene transfer was found to change the expression pattern of the intermediate filaments, of the cytoskeleton in MDA-MB-231 cells, which underwent a basic structural change [20].

Expression of keratin 8 and keratin 18 is normally maintained in breast carcinomas, whereas expression of other keratin family members is frequently lost [21]. The lack of correlation between CK8/18 and clinicopathologic and prognostic parameters might derive from an incomplete characterization of immunohistochemical expression of this marker. Our results suggest the maintenance of CK8/18 expression in most of the breast cancer cases studied but with a rearrangement of filament, which could be responsible for the three different expression patterns described in this study.

Based on previous report concerning the existence of a high clinical relevance in differentiating invasive ductal carcinoma subtypes, Walker LC *et al.* [22] reported two main groups, defined by heterogeneous or uniformly positive expression of KRT8/18 for G3-invasive ductal carcinoma of the breast no special type. In addition, they found 38 genes differentially expressed between these two classes included ERBB2, KRT8, and six other genes previously associated with ERBB2-positive or luminal phenotypes. Real-time polymerase chain reaction analysis revealed two molecularly defined clusters that aligned with the KRT8/18 staining phenotypes.

Association between aberrant granular pattern and overexpression of HER-2/neu oncoprotein, known as therapeutic target in breast cancer, needs future studies to identify if these two markers have mutual influence to each other.

✉ Conclusions

For a better characterization of the CK8/18 immunohistochemical expression in breast tumors, we suggest a score, which include three different expression patterns for each type of breast tumor. Previous studies together with our finding strongly support the use of CK8/18 expression for molecular classification of breast cancer.

Acknowledgements

This work was supported by Cp. no. 96/2007 Research Grant of Romanian Ministry of Education and Research.

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Received: February 20th, 2008

Accepted: October 15th, 2008