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ORIGINAL ARTICLE

Immunostaining of ΔNp63 (using the p40 antibody) is equal to that of p63 and CK5/6 in high-grade ductal carcinoma in situ of the breast

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Abstract As a result of breast cancer screening programs, high-grade ductal carcinoma in situ (DCIS) of the breast is diagnosed more often. Frequently, a DCIS diagnosis can only be made using immunohistochemical stains to visualize the myoepithelial layer in order to assess microinvasion. Standard markers for myoepithelial cells are CK5/6 and p63. An isoform of the latter, ΔNp63, is recognized by a recently developed antibody, p40. Here, we compare the standard myoepithelial markers CK5/6 and p63 with p40. We immunostained full sections of tissue samples of 35 high-grade DCIS and compared the staining pattern of CK5/6, p63 and p40 in tumour tissue and in normal glands. Staining patterns of myoepithelial cells for p63 and p40 were similar in terms of the percentage of stained nuclei. In all cases, p63 was strongly expressed, while this was the case for p40 in 31 (89 %) and moderately in 4 (11 %) cases. All but one case (97 %) showed a similar percentage of stained myoepithelial cells in comparing CK5/6 and p40 staining. CK5/6 expression was heterogeneous and strong/moderate/weak in 60, 34 and 6 % respectively. Compared to surrounding normal glands, staining of myoepithelial cells for all three markers in the neoplastic lesion was attenuated. In high-grade DCIS, p40 staining is highly specific for myoepithelial cells. Its staining pattern and intensity are equal to p63, which opens up its use for daily practice. Staining with p40 is less heterogeneous than that for CK5/6.

Keywords p40 · p63 · CK5/6 · DCIS · Myoepithelial cells

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Introduction

High-grade ductal carcinoma in situ (DCIS) of the breast is a precursor lesion for invasive breast cancer [1–3]. Since the introduction of breast cancer screening programs, DCIS is diagnosed more frequently and now accounts for approximately 20 % of all screening-detected breast malignancies [4], whereas in symptomatic cancers, it is found in 5 % of all cases [5]. DCIS originates in the epithelium of the ducts of the lobular system of the breast. It is composed of monotonous neoplastic cells with cytological atypia ranging from mild to severe. High-grade DCIS exhibits marked cytological atypia with or without necrosis [6]. Approximately one third of these lesions eventually progress to invasive carcinoma [7, 8]. The type of surgical treatment depends upon the extension of the lesion [9]. Breast conserving surgery can be followed by local radiotherapy [10].

Given the increased frequency of DCIS cases, surgical pathologists are now faced more often with the question whether a malignant epithelial proliferation is still confined to the intraepithelial compartment or expands beyond, justifying a conclusion of microinvasion. Invasive carcinoma arising from DCIS is diagnosed once small clusters of atypical cells are found beyond the myoepithelial layer of the ductal lobular unit [6]. Robust additional immunohistochemical (IHC) staining for myoepithelial cells can be very helpful in establishing a final diagnosis. CK5/6 is a widely used myoepithelial marker, which has as advantage that it usually does not stain neoplastic

epithelium [11]. It is expressed in basal-like carcinoma and its precursor lesions, which are characterized by poor clinical outcome [12]. The p53 homologue p63 is another routinely used marker and provides crisp nuclear staining of myoepithelial cells. Both CK5/6 and p63 immunostains require careful observation because staining intensity of myoepithelial cells in a context of neoplasia is weaker than staining of myoepithelial cells in normal tissues [13]. Recently, a new antibody that recognizes the p63 isoform ΔNp63 (p40) has become commercially available. So far, it has shown promising results as basal cell marker in the prostate as well as in the breast [14, 15]. It outperforms p63 in the detection of squamous differentiation in lung cancer, and it is slightly more specific for a diagnosis of prostate carcinoma, as fewer cases are false-positive [14, 16]. As yet it is unclear whether p40 provides the same diagnostic reliability as p63 and CK5/6, especially as staining of the myoepithelial layer is heterogeneous. We therefore compared in high-grade DCIS staining of myoepithelial cells for the markers p40, p63 and CK5/6.

Methods

Full sections of paraffin blocks from 35 cases of high-grade DCIS, 33 resection and 2 biopsy specimens were stained using an automated immunostainer (CK5/6, p63: Autostainer 480, Medac, Germany; p40: Ventana, Roche, Basel, Switzerland). After pretreatment with citrate buffer ph6, mouse monoclonal antibodies against CK5/6 (dilution 1:200) and p63 (clone: 4A4, dilution 1:400) (Dako, Hamburg, Germany) were used. Visualization was achieved with HRP-Polymer Detection Kit (Medac, Wedel, Germany). To stain the ΔNp63 isoform of p63, the p40 mouse polyclonal antibody (Zytomed, Berlin, Germany; dilution 1:100) was used after pretreatment with Cell Conditioning 1 (Ventana, Roche, Basel, Switzerland). UltraView Universal DAB Detection Kit (Ventana, Roche, Basel, Switzerland) was used for visualization. Staining intensity was semiquantitatively evaluated for CK5/6 by simple grading (0, 1+, 2+, 3+). When more than 1 % of epithelial cells were stained with CK5/6, the case was considered to be CK5/6 positive. For p63 and p40, a semiquantitative immunoscore was used, based upon the percentage of stained cells graded as 0 (staining of 0–10 % of the cells), 1 (10–50 %), 2 (50–75 %) or 3 (>75 %) and staining intensity as 0 (no staining), 1 (weak staining), 3 (moderate staining) or 4 (strong staining) The final score was % score × intensity score. Staining intensity and pattern were evaluated both in normal and malignant tissues. Statistical analysis was performed using SPSS 22.0 (IBM, New York, USA). .

Results

Age of our (female) patient cohort ranged between 26 and 85 years (median 61 years). Of our DCIS cases, 18 (51 %) were diagnosed as invasive and 17 (49 %) as non-invasive. In all cases, the percentage range of p63-stained myoepithelial

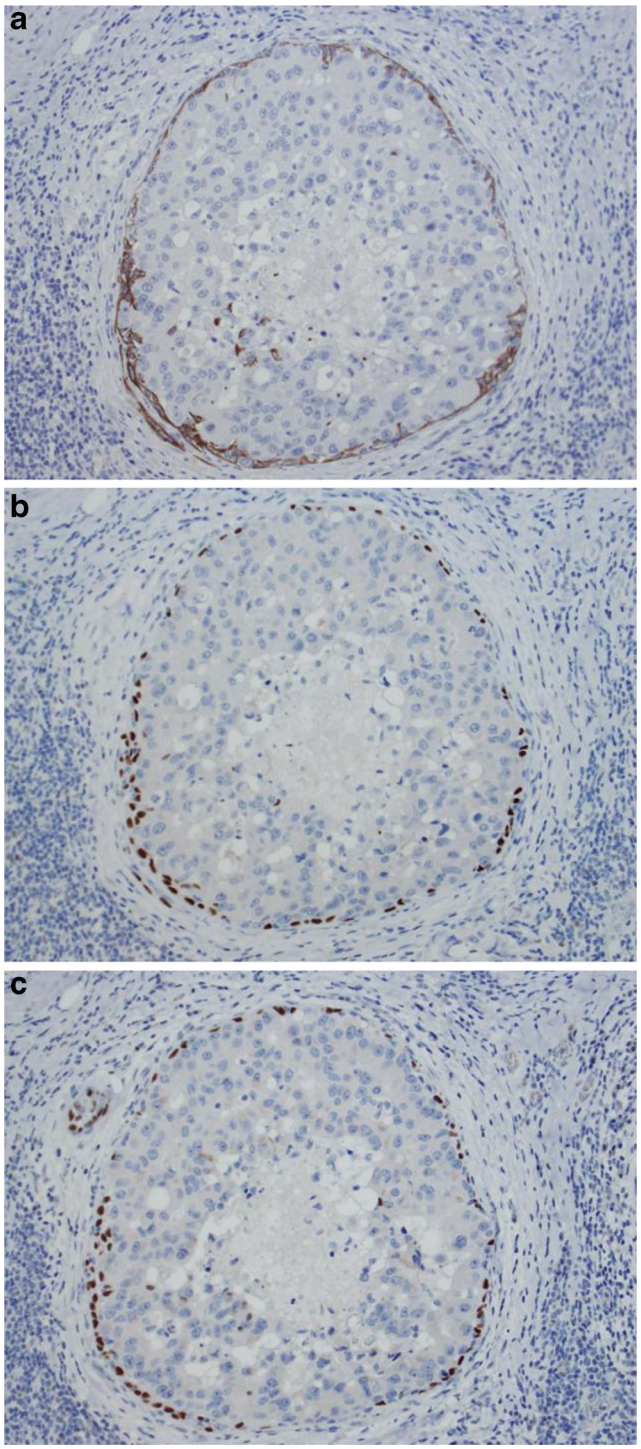

Fig. 1 Strong expression of CK5/6 (**a**), p63 (**b**) and p40 (**c**) in myoepithelial cells ×20

cells was similar to that of p40 (Figs. 1 and 2). In the majority of cases (97 %), the percentage of CK5/6-stained myoepithelial cells was similar to that of p63- and p40-stained cells (Figs. 1 and 2). Strong nuclear staining was noted in all p63 positive cases and in the majority of p40 positive cases (31; 89 %). Four cases (11 %) showed moderate staining intensity with p40. CK5/6 was expressed strongly in 21 (60 %) moderately in 12 (34 %) and weakly in 2 (6 %) cases. The number of cases in which invasion was detected was not different between the three markers.

Due to the slightly more aggressive tissue section pretreatment, p40 staining presented more artifacts, however without any deleterious effect on its diagnostic use.

Discussion

For a diagnosis of high-grade DCIS, often, additional immunohistochemical markers are used to stain the myoepithelial cell layer, in order to rule out or to confirm microinvasion. Established markers are CK5/6 and p63, but CD10 and smooth muscle actin (SMA) have also been used. Others [17] have not found CD10 to be a reliable marker, which corresponds to our experience. SMA staining has limited specificity since SMA is also expressed by myofibroblasts and capillaries [18]. Likewise, the 34βE12 antibody that detects high molecular weight cytokeratins 1/5/10/14 stains a large proportion of high-grade DCIS, and therefore, it is less specific than the antibodies we tested in this study [19]. Another basal-type cytokeratin, CK14, is also a highly sensitive marker of myoepithelial cells [20]. In view of our experience with CK5/6 immunohistochemistry, we used CK5/6 staining as reference in this study.

Two different isoforms of the p63 protein exist, TAp63 and ΔNp63, as a result of two different promoters in the *p63* gene [22]. The monoclonal antibody 4A4, which we used to stain p63, detects a shared domain in both isoforms, whereas p40, a rather novel antibody against the p63, only marks the ΔNp63 isoform [23]. As the staining pattern of both antibodies is virtually identical, we conclude that the ΔNp63 isoform is the predominant isoform in breast myoepithelial cells. We compared staining patterns of CK5/6, p63 and p40 in myoepithelial cells surrounding high-grade DCIS. We confirm that p63 is a reliable myoepithelial marker with a homogenous staining pattern [21]. The specificity of p40 for myoepithelial cells is similar to that of p63, and in most cases (89 %), nuclear staining was equally strong as seen for p63, even though the weaker staining in four cases did not hamper identification of the myoepithelial layer. Therefore, we conclude that p40 as myoepithelial marker in breast biopsies provides the same diagnostic accuracy as p63. In comparison with staining for CK5/6, p40 is more robust as it is less heterogeneous.

The majority of analysed DCIS were from resection specimens because our intention was to provide evidence of the reliability p40 as a myoepithelial marker. We also noted crisp nuclear staining in the few biopsy specimens included in the study, which provides a rationale for analysing p40 in a larger

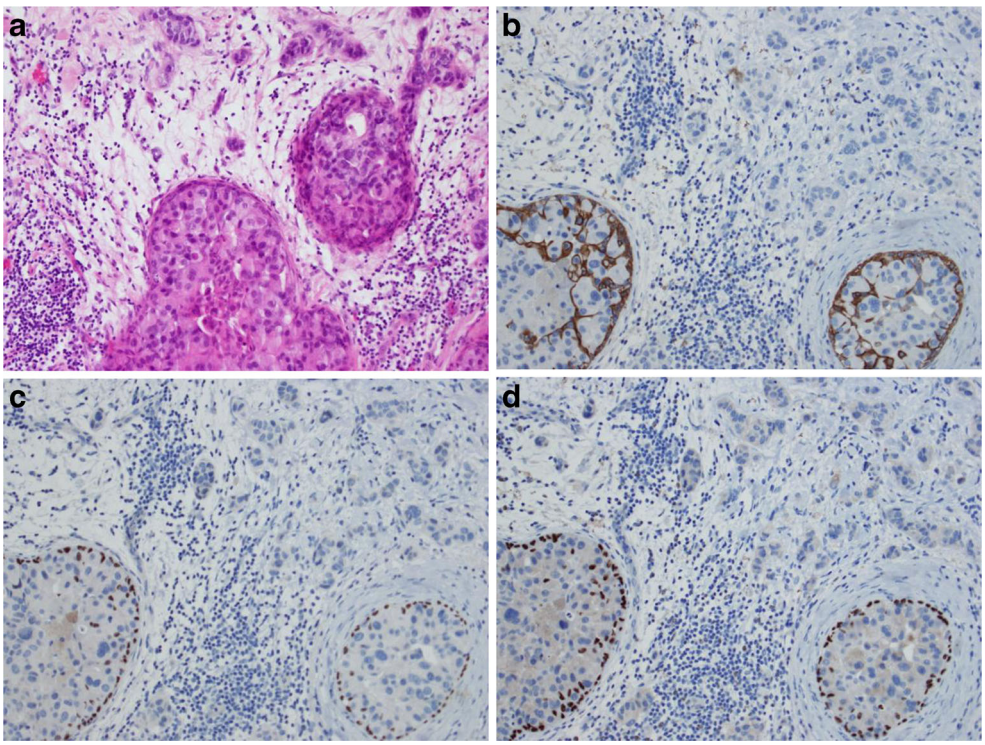

Fig. 2 DCIS and invasive tumour, HE (**a**). Strong expression of CK5/6 (**b**), p40 (**c**) and p63 (**d**) in myoepithelial cells

cohort of breast biopsies. In our experience, tissue fixation of breast biopsies is usually better than that of resection specimens, and therefore, p40 should work very well in those too.

All three myoepithelial markers revealed attenuated staining of myoepithelial cells surrounding neoplastic glands in comparison with that in surrounding normal tissue. We speculate that this might be a sign of tumour progression, since myoepithelium seems to function as a suppressor of invasion through paracrine effects on cell cycle progression, cell migration and invasion [24–26].

CK5/6 positive neoplastic cells are rarely encountered according to a recent publication in which this was found in 15 of 146 DCIS [27]. Although does constitute a diagnostic pitfall, the marked nuclear atypia in high-grade DCIS should prevent the pathologist from making an erroneous diagnosis of usual ductal hyperplasia (UDH).

In summary, p40 is a novel marker of breast myoepithelial cells with a performance similar to that of p63 and slightly better than CK5/6. However, it does not appear to provide a clear advantage over the commonly used p63.

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Conflict of interest The authors declare no conflict of interests.

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