The added value of SOX10 immunohistochemistry to other breast markers in identifying cytokeratin 5 positive triple negative breast cancers as of mammary origin.

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ABSTRACT

Aims: Triple negative breast cancer (TNBC) represents a specific group that lacks the expression of estrogen receptors, progesterone receptors, human epidermal growth factor receptor-2, and might also lack the expression other breast markers like GATA3, mammaglobin (MGB), GCDFP15 (growth cystic disease fluid protein 15) and NYBR1; when this occurs, proving the breast origin of a metastasis is a challenging task. In the present study we assessed the added value of SOX10 immunohistochemistry to known GATA3, MGB, GCDFP15 and NY-BR-1 statuses in a series of CK5 positive primary TNBCs.

Methods: Tissue microarrays (TMAs) were made from the formalin-fixed and paraffin-embedded blocks of 120 TNBCs and 3-4-micrometer-thick sections were immunostained for SOX10. The cut-off for a positive reaction was at least 10% of tumor cells staining.

Results: In our cohort, SOX10 positivity was seen in 82/119 cases, 61, 74, 76 and 82 all of which were GATA3, MGB, GCDFP15 and NY-BR-1 negative, respectively. Of the SOX10 negative cases, 12 stained with at least another breast marker. Nevertheless, 26/119 (22%) cases remained negative with all markers assessed.

Conclusions: SOX10 proved to be the most commonly positive breast marker in our CK5 expressing TNBCs, but the other markers also had some additive value to SOX10.

Keywords: triple negative breast cancer, immunohistochemistry, SOX10, tissue microarray

INTRODUCTION

About 15% of breast carcinomas belong to the so called triple negative category (TNBC) lacking the expression of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor-2 (HER2).[1] Most (although not all) of these cancers are aggressive and may give rise to metastasis relatively early in the course of the disease.[2, 3] The expression of ER, PR, sometimes even HER2, or “breast markers” like GATA3, mammaglobin (MGB), GCDFP15 (growth cystic disease fluid protein 15) and NY-BR-1 can point to the breast origin of a metastasis, but TNBCs - by definition - lack the first three and might also lack the others.[4, 5] In a previous work, we identified GATA3 as the most gratifying breast marker, which could still be complemented by MGB and GCDFP15, with practically no added value of NY-BR-1. Acknowledging that neither of these markers are absolutely specific, we also suggested that only about half of cytokeratin 5 (CK5) expressing TNBCs could be proven to be of mammary origin with their help, therefore better or alternative markers would be useful in clinical practice.[5]

SOX10 is a transcription factor involved in neural crest differentiation.[6] Accordingly, SOX10 positivity can be seen in melanoma, nerve sheath tumors,[7-10] and is also expressed in myoepithelial cells in the breast.[11] SOX10 positivity has been described in salivary gland and cutaneous adnexal gland tumors, as well.[12, 13] As many TNBCs are basal-like on the basis of gene expression profile,[14] and show CK5 and/or epidermal growth factor receptor (EGFR) positivity, and classify as basal-like on the basis of an immunohistochemistry (IHC) based surrogate classification proposed by Nielsen and colleagues,[15] it is not surprising that these cancers may also stain for another myoepithelial marker, SOX10. Indeed, SOX10 has been reported to be positive in 40% to 70% of TNBCs,[16, 17, 4, 18] and may be positive even in GATA3 negative cases.[18] In the present study we assessed the added value of

SOX10 IHC to GATA3, MGB, GCDFP15 and NY-BR-1 IHC in a series of CK5 positive TNBCs.

METHODS

A series of CK5 positive TNBCs previously characterized by GATA3, MGB, GCDFP15 and NY-BR-1 IHC in a previous analysis was used for SOX10 IHC. [5] The tumors were assessed in tumor microarrays (TMAs) being represented by dual 2-mm-diameter tissue cores; the details of TMA building were reported earlier. [5]

Briefly, the tumors were derived randomly from patients operated on and diagnosed with TNBC at the Bács-Kiskun County Teaching Hospital, Kecskemét between August 2005 and August 2015. The surgical specimens were fixed in 10% neutral buffered formalin for at least 24 hours. The TMAs had been constructed from archived paraffin-embedded blocks using a TMA builder device (Histopathology Ltd, Pécs, Hungary), with each TMA incorporating 20 tumor tissue cores. The TMA blocks were stored at room temperature, similarly to other paraffin blocks. 3-4-micrometer-thick sections were cut for SOX10 IHC using a monoclonal mouse antibody specific for an epitope mapping between amino acids 2-29 at the N terminus of SOX-10 of human origin (Santa Cruz Biotechnology, Inc., Dallas, TX). The antibody was used with 1:500 dilution for 30 minutes incubation period and pretreatment was performed at pH 9.

TMAs were scanned and the proportion of positive cells was independently evaluated on the digital slides by the authors, and the few discrepant cases were reassessed by consensus on the original slides. Rate, localization and intensity were registered in all cases.

The data for GATA3, MGB, GCDFP15, NY-BR-1 were taken from a previous analysis.[5]

The institutional ethical committee of the Bács-Kiskun County Teaching Hospital of the University of Szeged was consulted and approved this non-interventional retrospective study. The institutional data safety manager also gave approval for this study not requiring patients' identity related data. The study was finally approved by the ethical committee of the Albert Szent-Györgyi Medical Center of the University of Szeged.

RESULTS

Of the 120 TNBCs represented in the TMA cores, 119 could be assessed for SOX10 staining. SOX10 staining was generally a nuclear staining occurring in <1% to 100% of tumor cells (Figure 1), therefore two different cut-offs for positive staining were evaluated. With a positivity threshold of >1 % and ≥10%, 93 and 82 were defined as positive. Because the proportion of cases with 1-10% staining was relatively low, and the cases are less easy to pick up, the greater threshold was used for further analysis.

Of the 94 GATA3 negative cases, 61 cases were positive with SOX10. Similar results were observed with the other breast markers. 74 out of 104 MGB negative cases, 76 out of 109 GCDFP15 negative cases and 82 out of 117 NY-BR-1 negative cases stained positive with SOX10. Our series included 78 cases that were negative with all the previously tested markers, 52 of which were identified as positive with SOX10, still leaving 26 (21.8 %) as breast marker negative. The sequential hierarchical staining for breast markers from the most commonly positive to the least commonly positive is shown in Figure 2.

Mutual staining figures of pairs of breast markers are shown in Table 1.

Any staining	SOX10+	SOX10-	GATA3+	GATA3-	MG+	MG-	GCDFP-15+	GCDFP-15-
GATA3+	20	3						
GATA3-	61	33						
MG+	7	6	2	11				
MG-	74	30	21	83				
GCDFP-15+	5	4	2	6	2	6		
GCDFP-15-	76	33	21	88	11	98		
NY-BR-1+	0	2	0	1	0	1	1	1
NY-BR-1-	82	35	23	93	13	103	8	108

Table 1 Pairs of breast markers and their expressions in the tumors investigated

Figure 3 and Supplementary Table 1 illustrate the proportion of single and multiple marker expression in the series with all staining combinations experienced.

CONCLUSIONS

The expected lifetime risk of developing cancer in women is around 1 out of 3,[19] making the possibility of developing multiple primary cancers a real possibility and giving emphasis to the distinction between metastasis of a known primary tumor and an independent second primary cancer. TNBCs represent a minority of breast cancers, but as triple negative no special type carcinomas are often of poor prognosis, metastases may occur at a relatively higher rate. Owing to their phenotype overlapping with myoepithelial differentiation, TNBCs may show variable histologies, including spindle cells (sarcomatoid appearance), squamous metaplasia and other rarer (e.g. sebaceous, chondroid or osseous) metaplasias, making their recognition as metastatic breast carcinomas at a metastatic site more difficult. This is why IHC markers supporting the breast origin of TNBCs is important.

In our previous study, by using GATA3, MGB, GCDFP15 and NYBR1 we came out with an algorithmic value of these “breast markers” in CK5 expressing TNBCs believed to represent basal-like breast carcinomas, where GATA3 was the mostly expressed marker. Our results suggested that only about half of these cancers could be classified as of mammary origin on the basis of these four markers.[5]

In 2013 Cimino-Mathews et al published the first study investigating the utility of SOX10 IHC labeling in TNBC and metaplastic breast carcinoma cases. TMA blocks from 168 primary breast cancers were investigated; 40% showed positivity with SOX10, these were primarily basal-like unclassified TNBCs and metaplastic carcinomas. 66% of TNBCs but only 5% of luminal and HER2 positive cases were positive with SOX10.[16]

Nelson and coauthors reported about promising SOX10 results. TMA blocks were made from 26 patients' samples. 38% stained positively with SOX10, while no cases were positive with ER and HER2. A retrospective study was also performed in metastatic carcinomas of possible breast origin, and 57% of cases were labeled with SOX10. All SOX10 positive cases were confirmed to be negative with ER. Nelson and coauthors recommended the use of SOX10 in metastatic cases from unknown primary tumors to prove their melanoma or TNBC origin.[20]

Since 2017 several research groups started to investigate SOX10. Al-Zahrani and coauthors compared its application with androgen receptor (AR). AR staining was positive in 95% of cases, mostly along with HER2 or ER and PR positivity, but no special breast cancer subtype could be identified on the basis or AR staining alone. SOX10 proved to be positive in one third of cases that were triple-negative.[21]

Tozbikian examined the IHC profile of 57 TNBC cases. 82% showed positivity with GATA3, 58% with SOX10 and 25% with AR. 95% proved to be positive with either GATA3 or SOX10, and 46% showed dual positivity; 80% of GATA3 negative cases were SOX10 positive. Their study concluded that while GATA3 was a more sensitive marker for TNBC cases, it is useful to add SOX10 to the IHC panel.[18]

Harbhajanka et al investigated 48 TNBC cases in TMAs, and SOX10 showed positivity in 37,5% of the cases. A negative correlation with AR positive molecular subtypes of TNBCs was observed, and a positive correlation was proved with WT1. However, no correlation was seen with the breast markers GATA3, MGB, GCDFP15 and basal-like subtype markers EGFR and CK5/6.[22]

The most comprehensive study is the one by Laurent et al, who reported their results about SOX10, GATA3, GCDFP15, AR and MGB in 207 metastatic TNBC cases and compared them with 152 primary lung adenocarcinomas. SOX10 showed the best sensitivity (62.3%)

and specificity (100%) in comparison with GATA3 (30.4% sensitivity and 98.7% specificity), GCDFP15 (20.8% sensitivity and 98% specificity), MGB (38.2% sensitivity and 81.6% specificity) and AR (30% sensitivity and 86% specificity). 6.3% of TNBC cases had no reaction with any of the above mentioned markers.[4]

This year Qazi et al suggested the combined use of SOX10 and GATA3 in cases of low ER expression and reduced GATA3 intensity. The study included 246 patients' samples as TMA blocks containing both ER positive and negative cases. Overall, 93%, and of TNBC cases, 63% showed positivity with GATA3; in parallel 15% of all and 74% of TNBCs were positive with SOX10. Less than 1% had no reaction with either antibody and only 3% of ER positive cases were SOX10 positive.[23]

In our current study, by adding SOX10 immunohistochemistry to our previous "breast marker" panel, there was an improvement in identifying CK5 expressing TNBCs as of mammary origin, and the algorithm could be changed substantially (Figure 2). Using the 10% cut-off for SOX10 positivity, 68.9% (95% CI: 59.8-77.1) of the cases were found to be SOX10 positive and 9.3% of cases were positive only with the previously used markers, which proves the added value of GATA3, MGB, GCDFP15 and NYBR1. 21.8% of the cases remained negative with all "breast markers", suggesting that negativity of all the examined markers doesn't securely exclude mammary origin. These results are in keeping with former reports [4, 23], and suggest that SOX10 is probably the best "breast marker" of TNBCs, followed by GATA3. Minor discrepancies in the proportion of cases staining and the value of MGB versus GCDPF15 may stem from our cohort being restricted to CK5 expressing (and most likely basal-like) TNBCs, whereas others also included apocrine TNBCs which are expected to be positive with GCDFP15 (also an apocrine marker) more commonly. In 41% of the cases in our study, the staining observed was focal, in these, the application of the TMA technique can be a limitation.

Based on our data, SOX10 proved to be the most sensitive breast marker in CK5 expressing TNBCs, likely to correspond to basal-like TNBCs on the basis of the IHC based surrogate classification. With the additive value of GATA3, MGB, GCDFP15 and NY-BR-1, more than three quarters of the investigated 119 cases could be identified as breast cancers. With the joint use of SOX10, GATA3, MGB and GCDFP15 78,2% (95% CI: 69,7-82.2) sensitivity was achieved. We propose SOX10 as first line approach to identify TNBCs, with the addition of GATA3, MGB and GCDFP15 for the negative cases; NY-BR-1 has little added value in this context.

COMPLIANCE WITH ETHICAL STANDARDS

The institutional ethical committee of the Bács-Kiskun County Teaching Hospital was consulted and approved this non-interventional retrospective study, which was also approved by the Regional Ethical Committee of the Albert Szent-Györgyi Medical Center of the University of Szeged.

CONFLICTS OF INTEREST STATEMENT

No editorial or financial conflicts of interest exist for this submission.

CONTRIBUTIONS

Concept and design – Anita Sejben, András Vörös, Gábor Cserni

Evaluation of immunostained slides - all authors

Consensus on near cut-off staining cases – all authors

Search and evaluation of references – all authors

Drafting the manuscript – Anita Sejben, András Vörös, Gábor Cserni

Approval of final manuscript – all authors

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FIGURE LEGENDS

Figure 1 Examples of SOX10 immunostaining. A: diffuse strong nuclear staining (x20); B: partial staining in more than 10% of the cells with moderate intensity (x20); C: Over 10% of nuclei staining weakly (x40); D: Less than 1% of nuclei staining (x40).

Figure 2 Hierarchical labeling of the tumors with SOX10, GATA3, MGB, GCDFP15 and NY-BR-1 as “breast specific” markers

Figure 3 Number of cases demonstrating a given pattern of breast marker expression in the series investigated