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Research Article

Association of SOX10 Immunohistochemical Expression with Triple Negative Breast Cancer in Karbala City

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Abstract

Background: Triple-negative breast cancer (TNBC) is a specific group of breast cancer that is known for its aggressiveness and the lack of expression of estrogen (ER), progesterone (PR), and human epidermal growth factor receptor2 (HER2) antibodies. The aim of this study is to assess the immunohistochemical expression of SOX10 in TNBC, its correlation with tumor grade, and other clinical and pathologic parameters.

Methods: A cross-sectional study which included thirty paraffin blocks of primary TNBC that were collected from the Karbala Teaching Center of Histopathology. For each of the collected blocks, two sections were taken. One was stained by hematoxylin and eosin and the other was stained by immunohistochemical method for SOX10.

Results: SOX10 reported positive in 43% of positive cases versus 57% of negative cases. This positivity was significantly correlated with pathological grade (p =0.012) and the pathological stage (p =0.038). A particular statistical significance was found in association with the lymph node group and distant metastasis (p= 0.039 and p=0.037, respectively). No significant statistical difference between SOX10 expression and the patient's age, tumor size, presence of Ductal carcinoma in situ (DCIS), and lymphovascular invasion.

Conclusion: The study proposed that SOX10 expression can predict worse disease outcomes regarding higher grades and stages.

Keywords: SOX10; triple negative breast cancer; immunohistochemistry.

Introduction

Breast cancer (BC) is the most common cancer across the globe exceeding the number of cases of lung cancer for the first time in 2020. It is the number one cause of death of women in underdeveloped countries [1]. In Iraq and according to the cancer registry section (Iraqi Cancer Board/ Ministry of Health), breast carcinoma is the most common malignant tumor in Iraqi women, accounting for approximately one-third of all registered female cancers [2]. The major groups of triple-negative breast cancer include basal-like BL1, and BL2 subtypes, and other groups (mesenchymal subtype, mesenchymal stem-like, immunomodulatory, and luminal androgen receptor subtypes) [3]. Most TNBCs are high-grade invasive ductal carcinomas (IDCs; also known as invasive carcinomas of no special type), which have pushing borders, marked nuclear pleomorphism, numerous mitoses, and often have geographic zones of necrosis, and brisk lymphocytic infiltrates [4]. The treatment and outcome of breast cancer are affected by many parameters including the age of the patient, tumor size, grade and the stage of the tumor, histologic type, status of hormonal receptors expression as estrogen, progesterone, and Human Epidermal growth factor receptor Type- 2 (HER-2/neu) receptors, BRCA gene status and Ki-67 proliferation marker [5]. The percentage of triple-negative breast cancers (TNBCs) is about 12-17% of breast cancers and the main characteristic that establishes its definition is the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [6]. TNBCs are known for their worse behavior. The number of cases seems to be higher in younger women, and those with African and Hispanic origin and association with BRCA1 gene expression [7].

SRY-related HMG-box 10 (SOX-10) is a transcription factor located on chromosome (22q13.1) that has particular importance in the development of the neural crest including Schwann cells and melanocytes [8]. SOX10 immunostain is

a helpful ancillary test due to its high sensitivity for melanocytic and peripheral nerve sheath neoplasms, and its role in distinguishing triple-negative breast carcinomas from gynecological carcinoma, cutaneous adnexal neoplasms, and salivary glands neoplasms from histological mimics [9]. SOX transcription factors get their name because these proteins share greater than, or equal to 50% similarity of their high-mobility-group (HMG) domain to the SRY gene [9]. There have been 20 different SOX genes discovered in mice and humans and these 20 genes are further subclassified into groups. SOX8, SOX9, and SOX10 belong to the SOXE subclass of the SOX family of transcription factors [9]. Together, the SOXE subclass is responsible for neural crest migration and differentiation, chondrogenesis, gliogenesis, and sex determination [10]. Regarding breast cancer, SOX10 shows higher expression in triple-negative breast cancer, particularly the metaplastic type. It can coordinate the state of the stem cells and mesenchymal cells in breast tissue [11]. In mammary epithelial cells, SOX10 has a main role in the promotion of epithelial-mesenchymal transition (EMT) [12]. The attempts to develop targeted therapy for one of the genes of SOX10 for metastatic breast cancer are ongoing [13].

The aim of this study is to assess the immunohistochemical expression of SOX10 in TNBC, its correlation with tumor grade, and other clinical and pathologic parameters.

Materials and Methods

Patients

A cross-sectional study included thirty paraffin blocks of primary TNBC, that were collected from the Karbala teaching center of Histopathology in the period from January 2023 to December 2023. The study included 17 breast cores and 13 excision specimens. Information regarding the stage of cases was obtained from the department of Oncology in Al-Hussein Medical City. Cases with available clinical data including, age, size, grade, and stage were included in this study. Biopsies with insufficient and poor-quality pathological material were excluded.

SOX10 measurement

For each of the collected blocks, two sections were taken. One was stained by hematoxylin and eosin and the other was stained by immunohistochemical method for SOX10. Grading was obtained for each case using the Nottingham grading system [14] and staging was done following the TNM staging system. For recognition of SOX10, we used a monoclonal rabbit antibody (Clone: EP268) manufactured by Pathnsitu, USA. Sections from normal brain tissue were used as a positive control. Technical negative controls were obtained by omitting the primary antibody for the marker under identical test conditions. Bimanual staining was achieved by making 5 micrometer sections for each of the blocks and applying each one on a positive charge dewaxing followed by the step of antigen retrieval by Citrate Buffer (Cat#PS007), and by using steam pressure for 20 minutes using PathnSitu's MERS (Multi Epitope Retrieval System) then letting the solution cool at the room temperature, immerse the tissue sections slides in distilled water prior to the primary antibody application by covering the tissue sections with primary antibody and the incubation time required is about 60 min at room temperature. For the interpretation of SOX10, it expresses its positivity in the nucleus and the percentage of expression should be more than 1% of the tumor cells to be considered positive as indicated by the manufacturer literature. The interpretation and level of expression of SOX10 were negative if nuclear expression (<1%), patchy if nuclear expression (1-10%), focal (10-70%), and diffuse in case of (70-100%) nuclear expression. The microscopic sections were captured by using an Olympus Dp72 microscope camera.

Ethical approval

The research followed the ethical standards of Helsinki and the study gained its approval from the Ethics committee of the College of Medicine at the University of Karbala (ethical no: 24-13 on 13 March 2024). This study was conducted as the Histopathology fellowship thesis in the College of Medicine at the University of Karbala.

Statistical Analysis

Statistical analysis was performed using SPSS V. 26 (statistical package for social sciences) using a t-test for two continuous variables and one-way ANOVA with a post hoc test. Results were expressed as meant slandered deviation. The differences were considered statistically significant when the P value was less than 0.05.

Results

All of the submitted cases were female of which 53% were equal or above the age of fifty. SOX10 reported positive in 13 (43%) as in Figures 1,2, and 3 in cases versus 17 (57%) negative cases (Figure 4). This positivity was significantly correlated with pathological grade (p =0.012) and the pathological stage (p =0.038). A particular statistical significance was found in association with the

lymph node group and distant metastasis (p = 0.039 and 0.037, respectively). Out of the 13 positive cases, nine cases were grade III the remaining four cases were grade II and none of the positive cases were grade I. The level of expression was found to be higher in higher-grade tumors with a more diffuse pattern of expression of SOX10 in higher-grade tumors (p =0.038) (Figure 1,2,3).

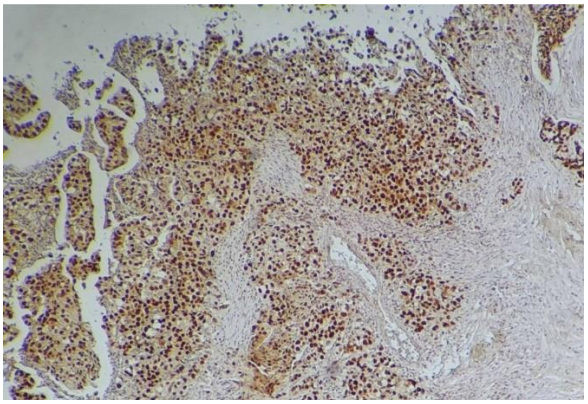

Figure 1. SOX10 expression in Invasive mammary ductal carcinoma Grade II diffuse pattern (100x).

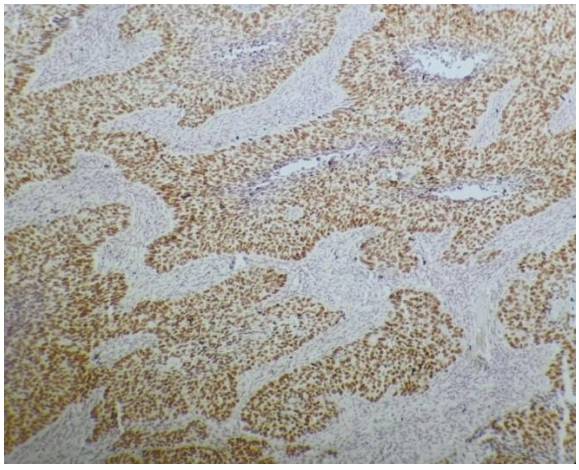

Figure 2. SOX10 expression in Invasive mammary ductal carcinoma Grade III diffuse pattern (100x).

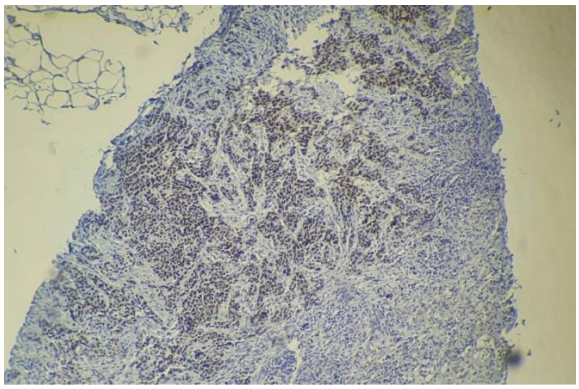

Figure 3. Invasive mammary carcinoma grade II with focal pattern of expression (100x).

Regarding the pathological stage, six positive cases were stage III and three positive cases for each stage IV and stage II, while there was a single positive case for stage I (Fig. 4). Regarding the lymph node group, seven positive cases were (N2) four positive cases were (N1) and only two positive cases were (N0). Distant metastasis was found in only three cases and all of them were positive for SOX10.

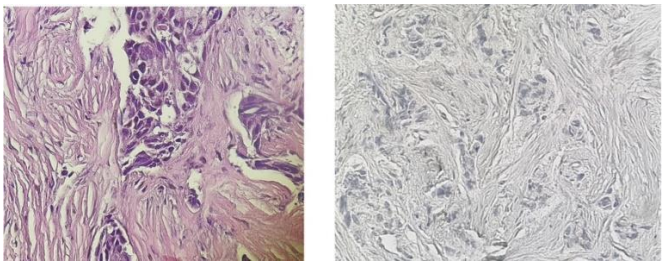

Figure 4. Invasive ductal carcinoma grade II H & E to the left and negative SOX10 to the right.

Age range from 23 to 76 years old with a mean age of 52 years. We set the age of 50 as a cut-point for age subgroup analysis. 16 (53%) of patients were fifty and above, and 14 (46%) were under fifty. The size range was from 1.2 to 6.6 cm with a mean size of 3 cm. We set 2 cm as the cutoff for size group analysis. The size of 11 cases (36.7%) was less than or equal to 2 cm and in 19 cases (63.3%) the size was more than 2 cm. DCIS components were present in ten cases (33.3%) and absent in 20 cases (66.7%). It was identified in five cases (16,7%), while it was absent in the remaining 25 cases (83.3%). No significant statistical difference between SOX10 was found between expression and the patient's age, tumor size, presence of Ductal carcinoma in situ (DCIS), and lymphovascular invasion (Tables 1 and 2).

Discussion

Our study on SOX10 IHC included 30 biopsy samples with primary TNBC. The expression of SOX10 in the total sample size was 43.33% (13 out of 30 cases). There are no other similar studies in Iraq. Linfang et al. (2020) [15] and Seemal et al. (2022) [16] detected positive SOX10 expression in 67.6% (48 out of 77 cases) and 67% (67 out of 100 cases) respectively. These variations may be due to the number of cases in each study. Another study by Rammal et al. (2022) showed that the positive rate of expression of SOX10 in the total sample size (n=157) was 67% [17].

The positive rate of SOX10 was significantly correlated with pathological grade (p =0.012), pathological stage (p =0.038), number of positive LN (p=0.039), and the presence of distant metastasis (0.037). Liu JL et al. (2022) [18] and Linfang et al. (2020) [15] are two Chinese studies that found concordant results regarding the significant association between high histologic grade and lymph node metastasis.

Table 1. SOX10 expression with clincopathologic parameters (n = 30)

Parameters		SOX10 expression			P- value
		Positive	Negative	Count of total (%)	
Age	<50y	6	8	14 (46%)	0.961
	>=50y	7	9	16 (53%)	
Grade	II	4	13	17 (57%)	**0.012**
	III	**9**	4	13 (43%)	
Stage	I	1	1	2 (6.7%)	**0.038**
	II	3	12	15 (50%)	
	III	**6**	4	10 (33%)	
	IV	**3**	0	3 (10%)	
LN group	N0	2	7	9 (30%)	**0.039**
	N1	4	8	12 (40%)	
	N2	**7**	2	9 (30%)	

Table 2. SOX10 expression with clincopathologic parameters (n = 30)

Parameters		SOX10 expression			P-value
		Positive	Negative	Total (%)	
T category	T1	5	6	11 (36.7%)	0.978
	T2	6	8	14 (46.7%)	
	T3	2	3	5 (17%)	
Distant metastasis	present	**3**	0	3 (10%)	**0.037**
	absent	10	17	27 (90%)	
Ductal carcinoma in situ	present	5	5	10 (33.3%)	0.602
	absent	8	12	20 (66.7%)	
Lymphovascular invasion	present	3	2	5 (16.7%)	0.410
	absent	10	15	25 (83.3%)	
Perineural invasion	present	2	0	2 (6.7%)	0.094
	absent	11	17	28 (93.3%)	

Moreover, the study by Linfang et al. (2020) showed concordant results regarding staging.

The Pakistani study by Seemal et al. (2022) showed an association with the tumor grade (p = 0.0006) only and no significant association with the tumor stage (p = 0.619) [16]. This may have something to do with the patient's demographics or differences in processing steps.

Results also revealed that there is no significant association between the marker expression and the age and the size of the tumor in the previously mentioned studies as well as in our study, as triple negative can be seen in both early and late-onset breast cancer. A higher level of positivity was found to be associated with higher-grade tumors (p =0.038) with a more diffuse pattern which was concordant with the study by Seemal et al. (2022). This may be attributed to a higher mutational burden in higher-grade tumors. The Chinese study by Linfang et al. (2020) included 71 cases of TNBC, 26 cases were grade II from which 13 cases were positive and the other 13 were negative. 45 cases were grade III of which 35 cases were positive and only ten cases were negative. Regarding the stage 28 cases were stage I-II from which 14 cases were positive and the other 14 were negative. 43 cases in stage III of which 34 were positive and only 9 were negative. Regarding lymph node stage 16 cases had 1-3 positive lymph nodes from which 14 cases showed positive SOX10 expression and 9 cases had more than or equal to 4 positive lymph nodes all of which showed positive SOX10 expression. The remaining 46 cases had no positive lymph nodes from which 29 cases showed SOX10 positivity. Seemal et al. (2022) study included 100 cases 32 cases were grade II and 68 cases were grade III. In the context of grade II cases, 18 cases were negative,6 showed patchy staining, 7 were focal, and 1 with a diffuse pattern. Of grade III tumors 15 were negative, 9 showed patchy staining, 25 cases were focal, and 19 cases showed diffuse patterns of staining.

In the current study, we found another association with distant metastasis (p =0.037) which supports the aggressive nature of triple-negative breast cancers. Further no association with the presence of DCIS or LVI. The previously mentioned studies didn't discuss these parameters and there were no published papers regarding the association of SOX10 expression with these parameters which require further studies of larger sample size to illustrate their rule if any can be found.

Conclusions

The study showed that higher positivity of SOX10

was considerably associated with worse outcomes of TNBC. This proposed that SOX10 expression is a possible prognostic marker for TNBC. The study also showed the clinical possibility of targeting the SOX10 gene for TNBC therapy. We recommend further studies with a larger number of cases as TNBC is widely heterogeneous including a vast spectrum of entities with marked molecular, transcriptional, histological, and clinical differences.

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