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Association of SOX2 expression with histopathological factors of invasive breast carcinoma in East Coast Malaysian women.

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Abstract – Background: Breast cancer is the most common cancer in women and is a leading cause of cancer-related mortality. The SOX2, having the ability of cancer stem cell self-renewal is responsible for the development of breast cancer. Objective: This study aimed to determine the frequency and gene expression pattern of SOX2 and its association with clinicopathological factors in invasive breast cancer in East Coast Malaysian women. Material and methods: The SOX2 expression was evaluated by immunohistochemistry on 100 samples of histologically diagnosed cases of invasive breast carcinomas, with known ER, PR and HER2 status, retrieved over 4 years period from January 2012 to December 2015. Positive samples were subjected to fluorescent in-situ hybridisation to determine the gene expression pattern. Result: The SOX2 expression was detected in 6% of invasive breast carcinomas, and all these cases were due to gene amplification. There was a significant positive correlation with tumour recurrence (**P**<0.001), large tumour size (**P**<0.001), lymphovascular invasion (**P**=0.006) and lymph node metastasis (**P**<0.01). The *SOX2* expression was not correlated with age group (**P =** 0.078), tumour grade (**P** = 0.465), ER positivity (**P** = 0.578), PR positivity (**P** = 0.578) or HER2 overexpression (**P** = 0.541). Conclusion: The SOX2 expression has a potential to be used as a prognostic marker in breast cancer in which the expression indicates potential for early recurrence and aggressive tumour behaviour. The SOX2 may also be considered as a target for immunotherapy to personalise the therapeutic strategies especially in preventing tumour recurrent.

Keywords: invasive breast carcinoma, SOX2, tumour recurrence.

1. Introduction

Breast cancer is the most common cancer worldwide with two million cases were diagnosed and 626,679 breast cancer mortality reported in the year 2018 by Globocan, WHO. A large increment in breast cancer mortality was also reported every year in Asia-Pacific region [18]. Breast cancer development involved many factors including genetics, hormonal and environmental factors. The ability of tumor cells to become resistant to adjuvant therapy [19] affects the prognosis of the patient and the mechanism is still poorly understood. The *SOX2* gene, located in chromosome 3q26.33, is a member of the SOX (SRY-related HMG box) family of transcription factors. The SOX2 has various modification function on the enzyme activity such as phosphorylation, sumoylation, acetylation, methylation and also glycosylation [20]. It is one of the tumor transcription factors for cancer stem cells and it has the ability to maintain differentiation, self-renewal capacity and the pluripotency of embryonic stem cells [7]. It is also one of the tumor markers for cancer stem cells (CSCs) which play a major role in tumorigenesis [17]. The oncogenic potential of SOX2 leads to the ability for tumor invasion and metastasis in breast cancer. The presence of CSCs in breast tumors is likely one

of the main reasons why current oncologic therapies are poorly effective in preventing tumour progression, metastasis and recurrence [13, 15, 2].

The *SOX2* gene is expressed in the early stage of breast tumours and shows increase transcriptional activity of the cells by modifying the CARM1 enzyme to maintain the pluripotent state that is essential for maintaining self-renewal of undifferentiated embryonic stem cells [21]. This ability contributes to the recurrence state of tumor. An 82% of *SOX2*-positive disease have the risk of recurrence compared to only 42% in SOX2-negative disease [3] and the *SOX2*- positive patients reverted much earlier than patients with the SOX2-negative disease. This finding shows that over-expression of SOX2 is involved in the tumorigenesis of breast cancer and is associated with disease progression and poor clinical outcome [14]. The current study aims to determine the frequency and gene expression pattern of SOX2 and its association with clinicopathological factors in invasive breast cancer in East Coast Malaysian women for prognostic prediction and towards personalizing the therapy for patients.

2. Materials and Methods

2.1 Design

This study was a cross-sectional study involving one hundred cases of female invasive breast cancer undergoing mastectomies and wide local excision surgery from January 2012 to December 2015 in Hospital Sultanah Nur Zahirah (HSNZ), Kuala Terengganu.

2.2 Objectives

The general aim of this study is to investigate the expression of SOX2 gene in invasive breast cancer cases in East Coast Malaysian women and to correlate the presence of SOX2 gene with the clinicopathological factors of breast cancer.

2.3 Sampling

Archived tissue slides stained with routine Haematoxylin-Eosin were retrieved from the Histopathology Unit, Pathology Department, HSNZ. Tumour were evaluated and classified using a modified Bloom and Richardson grading system and also classified according to their molecular subtypes for the purpose of this study. The molecular subtypes were divided into 4 groups as Luminal A, Luminal B, HER2 positive and basal-like.

2.3.1 Immunohistochemistry

Archived tissue blocks from the tumour were retrieved and sectioned into 2-3 micron thick. Immunohistochemical stain (SOX2) was prepared using standard streptavidin-biotin-peroxidase technique. Rabbit polyclonal anti-human SOX2 antibody, ab97959 ABCAM was used.

2.3.2 SOX2 interpretation

Staining were considered positive when there was a moderate or strong immunoreactivity of the nucleus of the tumour cells.

2.3.3 Flourescence in-situ hybridisation: Direct fluorescence in-situ hybridization (FISH) assay with a two-colour interphase was performed on the positive SOX2 cases by immunohistochemistry using DAKO method for Histology (FISH Accessory kit DAKO code K5799). The ABNOVA *SOX2*/CEN3q FISH probe (catalogue no. FG0074) was used.

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2.3.4 Histopathological data

Data on histopathological factors of patients' breast cancer including ER, PR and HER2 expression was obtained from HSNZ Laboratory Information System.

2.4 Statistical data analysis

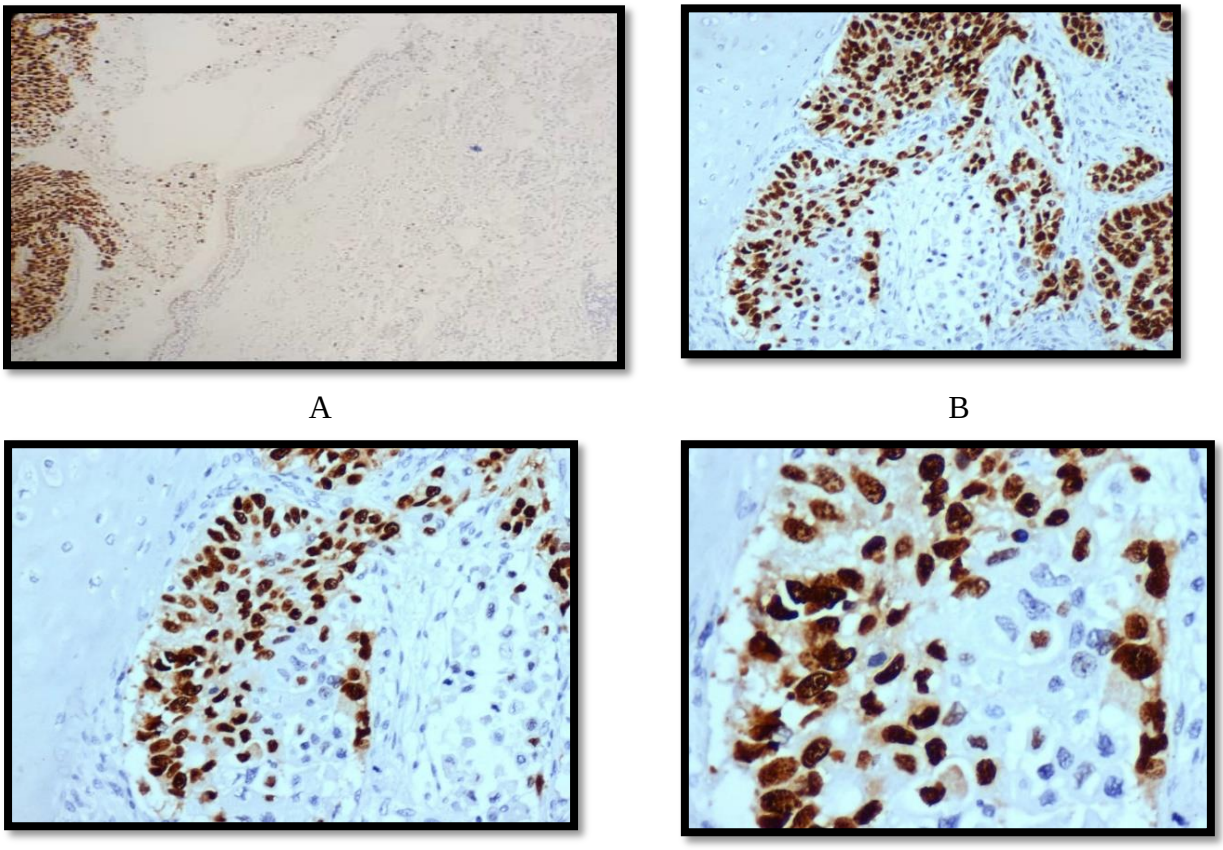
The data was analysed using statistical software SPSS for Windows, version 20.0. The association between categorical variables was tested using Fisher exact test. Factors with **P**<0.05 were considered significant. All tests were adopted by two-tailed and with a 95% confidence interval.

2.5 Ethical approval

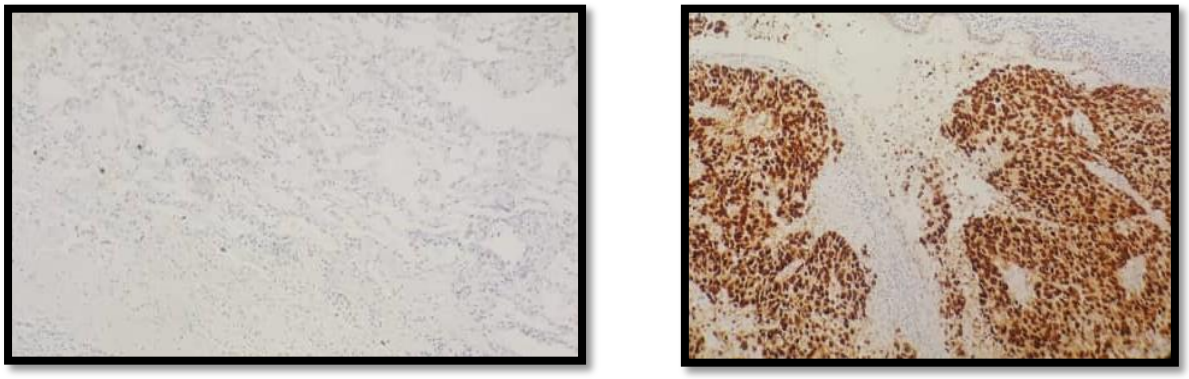
The study protocol obeyed the ethical guidelines of the Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-15-2345-28162) and was approved by the Malaysia National Medical Research Register.

3. Results

Six of 100 cases (6%) of invasive breast carcinoma were found to exhibit nuclear SOX2 expression. On the other hand, no SOX2 expression was detected in the benign tissue adjacent to tumour (figure 1). All six cases that exhibit positive nuclear SOX2 expression showed an amplified SOX2 gene when analysed by FISH study (figure 2).



A B C D



E F

Figure 1: A. SOX2 expression tissue with adjacent breast tissue. B. SOX2 expression in invasive breast carcinoma (x10 objective). C. SOX2 expression in invasive breast carcinoma (x20 objective). D. SOX2 expression in invasive breast carcinoma (x40 objective). E. Negative SOX2 expression in normal breast tissue (x10 objective). F. Tissue from positive control. (x10 objective)

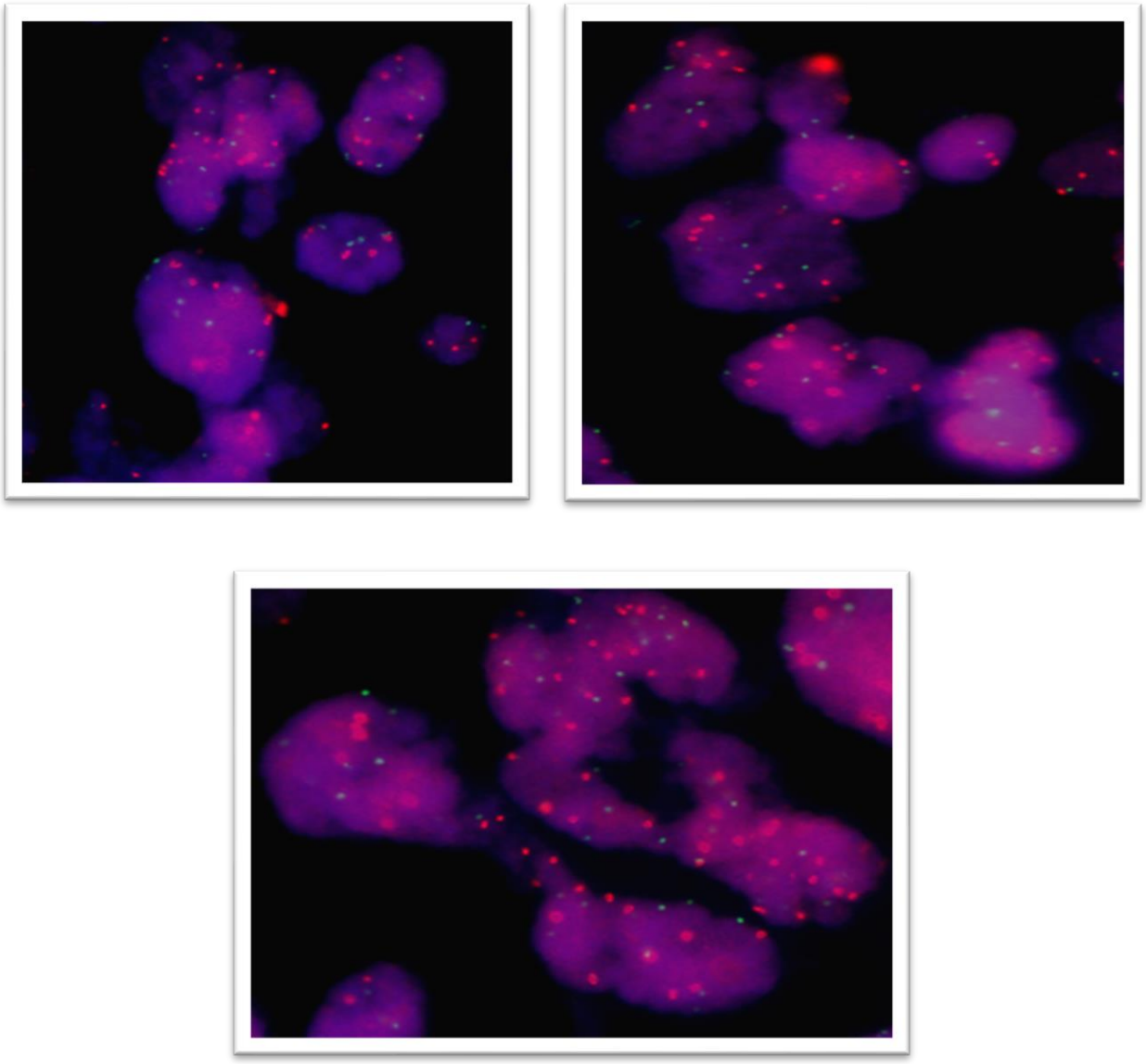


Figure 2: SOX2 amplification in invasive breast carcinoma assessed by fluorescence in situ hybridization. Red dot represents SOX2 gene and green dots for centromere.

Statistical analysis showed SOX2 positivity has a strong tendency to occur in the older group of patients (**P**=0.078) with mean age of cases was more than 50 years old (n=60) (table 1).The SOX2 expression is significantly associated with tumor size, lymphovascular invasion and lymph node metastasis (**P**<0.01). Furthermore, SOX2 was expressed more frequently in tumors of a higher histological grade. Four cases out of 54 cases (7.4%) from tumor grade 2 and two cases out of 40 cases (5.0%) from tumor grade 3 showed a positive SOX2 expression. However, we did not find their significant association statistically. Other than that, SOX2 nuclear expression in molecular subtype was found in two out of 38 cases (5.3%) of luminal A, in two out of 24 cases (8.3%) of luminal B and in two out of 23 cases (8.7%) of basal-like groups. None of the SOX2 expression was found from the HER-2 positive group. Similarly, we did not find their significant association statistically. Other than that, we also did not find a significant association between the SOX2 expression and other clinicopathological parameters such as tumor grading, hormone receptor (ER, PR) and HER2 status (table 2).

	***SOX2* expression**		
	Negative (N=94)	**Positive (N=6)**	**P value**
Age			
<50	40 (42.6%)	0 (0.0%)	0.078
≥50	54 (57.4%)	6 (100.0%)	

Table 1: Association between SOX2 expression with demographic data

	Negative (N=94)	**Positive (N=6)**	**P value**
Tumour size (cm)			
<5	53 (56.4%)	0 (0.0%)	0.001
≥5	41 (44.6%)	6 (100.0%)	
Histological Grade			
Grade 1	6 (6.4%)	0 (0.0%)	
Grade 2	50 (53.2%)	4 (66.7%)	0.465
Grade 3	38 (40.4%)	2 (33.3%)	
Molecular Subtype			
Luminal A	36 (38.3%)	2 (33.3%)	
Luminal B	22 (23.4%)	2 (33.3%)	0.677
HER2 +ve	15 (16.0%)	0 (0.0%)	
Basal-like	21 (22.3%)	2 (33.4%)	
Oestrogen receptor			
Negative	36 (38.3%)	2 (33.3%)	0.578
Positive	58 (61.7%)	4 (66.7%)	
Progesterone receptor			
Negative	36 (38.3%)	2 (33.3%)	0.578
Positive	58 (61.7%)	4 (66.7%)	
HER2			
Negative	57 (60.6%)	4 (66.7%)	0.541
Positive	37 (39.4%)	2 (33.3%)	

Lymphovascular Invasion			
YES	38 (40.4%)	6 (100.0%)	0.006
NO	56 (59.6%)	0 (0.0%)	
Lymph Node Metastasis			
0	30 (31.9%)	0 (0.0%)	
<4	28 (29.8%)	0 (0.0%)	0.012
>4	36 (38.3%)	6 (100.0%)	

Table 2. Associations between SOX2 expression with clinicopathological and immunohistochemical features in invasive breast carcinomas

However, SOX2 expression showed a positive significant association with tumour recurrence (**P**<0.001) (table 3).

	SOX2 expression		
	Negative (N=94)	**Positive (N=6)**	**P value**
Recurrent status			
Yes	21 (22.3%)	6 (100%)	<0.001
No	73 (77.7%)	0 (0%)	

Table 3: Association between SOX2 expression with tumour recurrence

4. Discussion

The data from previous researches suggested multiple roles of SOX2 in the development and its association with breast cancer. This is the first study conducted on East Coast Malaysian women to determine the frequency and gene expression pattern of SOX2 and its association with clinicopathological factors in invasive breast carcinoma cases. In our study, 6% of the invasive breast carcinoma cases were SOX2 positive detected from IHC staining in which all the cases were due to the SOX2 gene amplification as determined by the FISH study. This finding is lower compared to the incidence reported by the previous study in other populations. Positive expression of SOX2 in invasive breast carcinoma was recorded in 19%, 34% and 17% of the cases in study conducted in China [5], Egypt (Nehad and colleague) and Spain [7] respectively.

We found that the SOX2 expression was significantly associated with lymphovascular invasion and high number of lymph node metastasis (**P**<0.05). This finding is similar to other studies (Rodriguez-Pinilla S.M. et al., 2007 and Langerke et. al., 2011). These results indicate that SOX2 contributes to tumor progression and lymph node metastasis in breast cancer. The SOX2 nuclear expression was strongly associated statistically with the recurrence status of the breast cancer (**P**< 0.001). Similar result was also found by another study [3] in which they found 82% of SOX2-positive disease have the risk of recurrence compared to only 42% of SOX2-negative disease. Until now the events leading to breast cancer progression or recurrence within a variable time interval are not completely understood. The accurate prediction of recurrence or the development of metastasis will become a useful tool for oncologists to develop personalized treatment strategies and identifying high-risk patients. The SOX2 can be a new prognostic marker of early recurrence irrespective of other clinicopathological features.

Our study also found a statistically significant positive correlation of SOX2 with large tumor size of more

than 5 cm (**P**<0.001). Our findings are in agreement with another study [22]. The SOX2 has a good cooperation with B-catenin that control the regulation of cyclin D1 (CCND1) which promotes cancer cell regulation and tumourigenesis by accelerating the transition of the cell cycle and regulating the transcription in breast cancer [1]. The SOX2 also plays an important role in inhibiting the cell apoptosis and promoting cell proliferation in breast cancer as demonstrated by its ability to increase the population of S+G2/M phase cells and to decrease the G0/G1 phase population (Liu K. et al., 2013). In this study, positive SOX2 expression tends to occur in breast tumors of patients aged more than 50 years old although statistically, it is not significant (**P**=0.078). This is in line with the earlier report that suggested the SOX2 is expressed more in postmenopausal breast carcinomas compared to premenopausal patients [8].

We also noted that there was no significant association between the SOX2 expression and the tumor grade (**P**= 0.465). Nevertheless, patients with high-level of SOX2 expression was found in the groups with higher tumor grades, which were 7.4% from Grade 2 and 5.0% from Grade 3 tumors. This finding was similar to another study [8]. Other than that, we found no significant correlation between the expression of SOX2 with ER (**P**=0.578), PR (**P**=0.578) and HER-2 status (**P**=0.541). These findings were in agreement with previous reports of several studies [8, 22, 12, 5]. In addition, our study also demonstrated that there was no significant difference in the immune expression between basal-like type and non-basal-like type breast cancer. The molecular group subtype showed no significant association with the SOX2 expression (**P**=0.677). The luminal A cancer showed the lowest expression of SOX2 among all molecular groups (5.3%) compared to the luminal B cancers and triple negative subtype which showing only 8.3% and 8.7% positive SOX2 expression respectively. This result indicates that SOX2 expression does not discriminate amongst the molecular subtypes of breast cancer. This finding was in line with a previous study that found the SOX2 expression was across different breast cancer subtypes [11].

5. Conclusion

The SOX2 expression was associated with large tumor size, recurrences, lymphovascular invasion and a greater number of lymph node metastasis in invasive breast carcinoma. Therefore, the SOX2 expression has a potential to be used as a prognostic marker in breast cancers in which the expression indicates a poor prognosis. The use of SOX2 also may help to personalise the treatment strategies and identify high-risk patients. SOX2 can be a prognostic marker of early recurrence irrespective of other clinicopathological features.

6. Conflict of interest

The authors declare no competing interest for this study.

7. Acknowledgement

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