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SOX10 and Folate Receptor Alpha are Frequently Expressed in Triple Negative and Progesterone Receptor Negative Breast Cancers

Tacha D, Qi W, Yu C, and Hoang L; Biocare Medical, Concord, CA

Background

Specific biomarkers can be essential for developing effective treatments for aggressive breast cancers, especially triple negative subtypes, for which treatment options are limited. Folate receptor alpha (FRα), a critical membrane protein for DNA synthesis and cell metabolism, has been suggested to be involved with the transformation of breast cancer into aggressive subtypes; and FRα has been shown to be strongly associated with poor prognosis in triple negative breast cancers (TNBC) as well as estrogen receptor (ER) positive and progesterone receptor (PR) negative subtypes.[1-3]

SOX10 is a nuclear transcription factor that participates in neural crest development and in the differentiation of cells of melanocytic lineage. Data suggests that SOX10 may contribute in stem cell or progenitor cell maintenance.[4] Recently, SOX10 has also been documented in benign breast myoepithelial cells and is associated with aggressive breast cancers.[5, 6]

This is the first study to compare FRα and SOX10 and immunohistochemical profiles in breast cancers with emphasis on TNBC. The study will mainly focus on SOX10 as several studies have been previously published on FRα immunohistochemical profiles in breast cancer; however comparative data will be evaluated with SOX10.

Design

Paraffin blocks of a previous 189 case study of breast cancers that had been classified according to their ER/PR/HER2 and FRα immunohistochemical status were identified and sections were recut at 4µm. Tissue sections were deparaffinized and hydrated down to water. Slides were immersed in a modified citrate buffer solution and heated at 95°C for 40 minutes. A mouse monoclonal SOX10 [BC34] (Biocare Medical, Concord, CA) was titered and optimized at 1:100 and applied to tissue sections for 30 minutes, followed by a polymer detection system and stained with DAB. The SOX10 results were compared to the previous study set of ER/PR/HER2 and FRα stained slides. Cut-off values of >1% and >5% of tumor cells staining were adopted to determine positivity for ER/PR and FRα/SOX10, respectively. Only 3+ HER2 cases were deemed positive for the purposes of this study.

Results

SOX10 was expressed in nuclei in normal breast ducts and in breast cancers; FRα was expressed in cytoplasmic/cell membrane components of normal breast ducts and in breast cancers (Figure 1A-D). SOX10 achieved a sensitivity of 42.1% (8/19) in ER+/PR-/HER2- cases (Figure 2) and was negative in all ER+/PR-/HER2+ cases ($p<0.05$). FRα was positive in 7.6% (7/92) of ER+/PR+/HER2- cases and was negative in all ER+/PR+/HER2+ cases. Similarly, FRα stained 52.6% (10/19) of ER+/PR-/HER2- cases and was negative in all ER+/PR+/HER2+ cases ($p<0.005$). SOX10 and FRα were observed in 3.3% (1/30) and 20% (6/30) of HER2+ cases, respectively.

In ER-/PR-/HER2- (triple negative) cases, both markers were highly expressed with 40% (10/25) and 52% (13/25) positive cases with SOX10 and FRα, respectively; and 24% (6/25) of cases were positive with both markers.

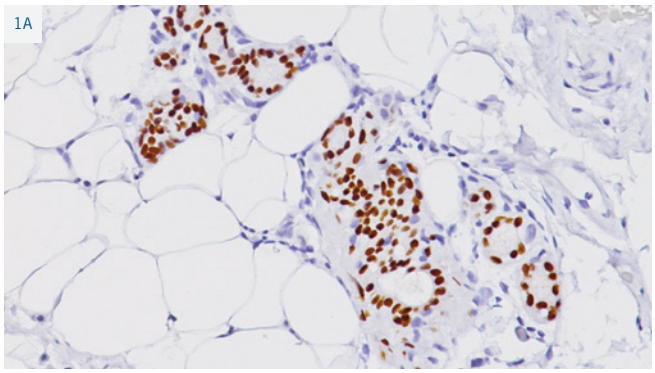
Table 1: SOX10 and FRα Expression in Breast Cancer Subtypes

ER/PR/HER2 Classification	% SOX10 (+)	% FRα (+)	% of co-expression of SOX10 and FRα
ER+/PR+/HER2+ (n = 13)	7.7% (1/13)	0.0% (0/13)	0.0% (0/13)
ER+/PR+/HER2- (n = 92)	6.5% (6/92)	7.6% (7/92)	2.2% (2/92)
ER+/PR-/HER2+ (n = 10)	0.0% (0/10)	50.0% (5/10)	0.0%(0/10)
ER+/PR-/HER2- (n = 19)	42.1% (8/19)	52.6% (10/19)	26.3% (5/19)
HER2+ (n = 30)	3.3% (1/30)	20.0% (6/30)	0.0% (0/30)
ER-/PR-/HER2- (n = 25)	40.0% (10/25)	52.0% (13/25)	24.0% (6/25)

As Presented at SABCS 2014, Poster Session 6 #13

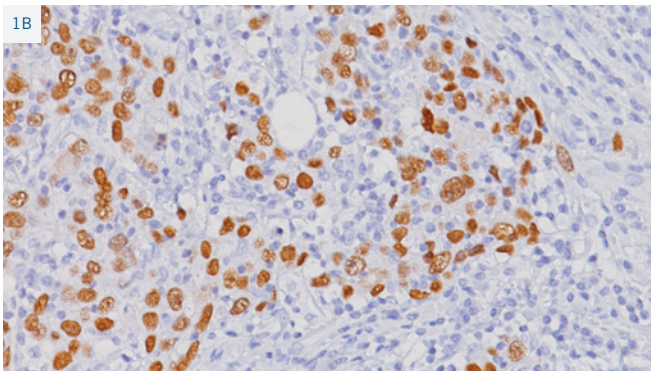
Figures

1A



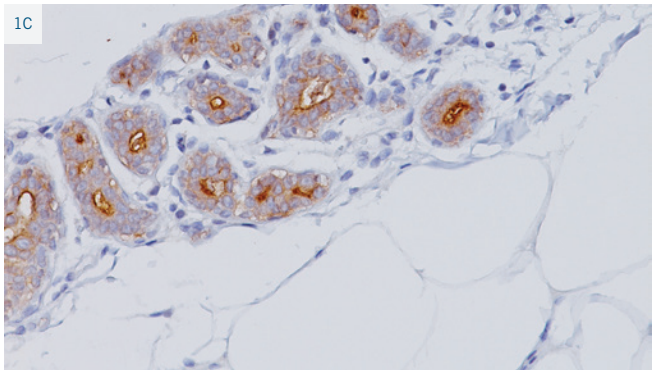
SOX10 stained in normal breast ducts

1B



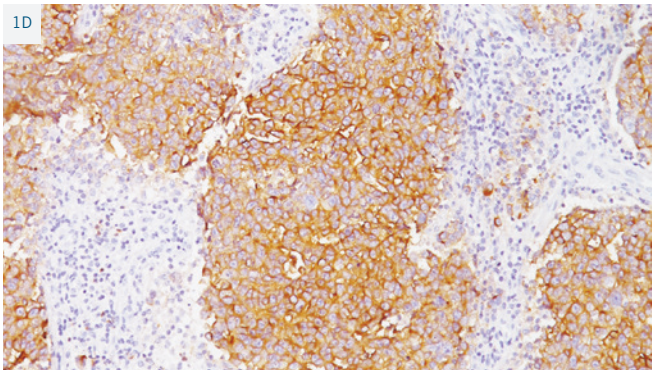
SOX10 stained in triple negative breast cancer

1C



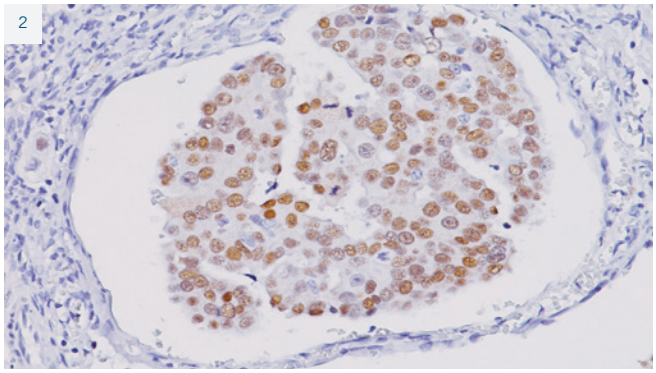
Folate receptor alpha stained in normal breast ducts

1D



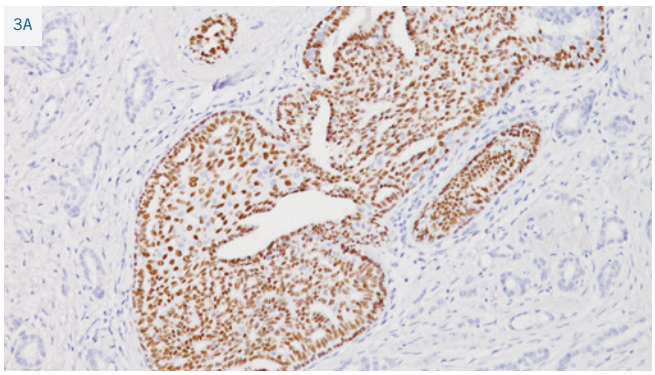
Folate receptor alpha stained in triple negative breast cancer

2



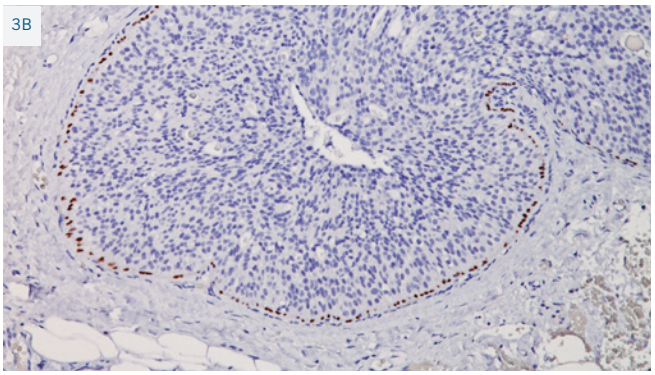
SOX10 stained in estrogen receptor positive & progesterone negative breast cancer

3A



SOX10 stained in ductal hyperplasia of the breast

3B



SOX10 stained in atypical ductal hyperplasia of the breast

Discussion

To date, SOX10 has been used primarily to support the diagnosis of melanoma in primary and metastatic sites including desmoplastic and spindle cell melanoma.[7] The implications of SOX10 expression in TNBC are not well understood and its biological significance remains unknown in breast cancers. Conversely, studies have demonstrated that a significant subgroup of ER and PR negative breast cancers, as well as TNBC frequently express FRα, and its expression is associated with worse clinical outcome.[1-3]

It was described in a recent work of Cimino-Mathews *et al* that SOX10 was expressed in breast cancers, mainly in triple negative cases; thus changing the previous perception that SOX10 was only expressed in melanoma.[5] In comparison, our study demonstrated similar results in TNBC, and interestingly, their study demonstrated low expression of SOX10 in 7.1% (1/14) of HER2 negative cases, which was comparable to our findings of 3.3% (1/30, Table 1). The significance of this finding remains unknown.

In our study, we observed SOX10 nuclear expression in myoepithelial glands in normal breast glands (Figure 1A), in breast ductal hyperplasia (Figure 3A), and in breast myoepithelial cells in atypical ductal hyperplasia (Figure 3B). Therefore, we concur with the Cimino-Mathews study that SOX10 expression in the basal-like and unclassified TNBC may support the concept that these neoplasms show myoepithelial differentiation.[5] Furthermore, Zhu YT *et al* demonstrated that peroxisome-proliferator-activated receptor-binding protein (PBP) is essential for the growth of Notch4-immortalized mammary cells by activating SOX10 expression, thus linking SOX10 to a potential molecular mechanism which regulates the growth of mammary stem/progenitor cells.[8]

It has been also suggested that SOX10 may play a role in stem or progenitor cell maintenance.[4] However; the mechanisms that maintain multipotency of stem cells are not fully understood. There is increasing evidence that cancer stem cells play a critical role in breast cancers and may be responsible for resistance during breast cancer therapy.[9, 10] CD271 (p75NTR), a nerve growth factor receptor, has been shown to be crucial in maintaining tumorigenicity and stem-like properties of melanoma cells.[11] Tomellini E *et al* determined that a shared set differentially regulated genes linked p75NTR directly to SOX10.[12] Civenni G *et al*, identified CD271-positive cells as melanoma stem cells and showed a high frequency of CD271/Sox10-positive cells correlated with higher metastatic potential and worse prognosis.[13] Therefore there is a potential connection of a SOX10 signature in the maintenance of breast cancer stem cells; and thus may be loosely extrapolated to propose that high SOX10 expression in ER+/PR- and/or TNBC could be an indicator of drug resistance and/or an aggressive phenotype in breast cancers. However, the further prognostic role of SOX10 and other related gene factors in breast cancers need to be addressed in future studies.

Finally, the observation of high expression of FRα and SOX10 in ER+/PR-/HER2- cases cannot be overlooked. Our study demonstrated that SOX10 and FRα was expressed in 42.1% and 52.6% of cases, respectively, and co-expressed in 26.3% of cases (Table 1). Studies have shown that ER+/PR- tumors are larger in size, exhibit a higher S-phase fraction, and are more likely to be aneuploidy, compared ER+/PR+ tumors. PR loss was identified in luminal B-type breast cancer subgroups and was at higher risk of relapse and death with HER-2-positive and HER-2-negative cases. Thus ER+/PR- are considered to be an aggressive subtype that may be similar to that of TNBC.[14, 15]

Conclusion

SOX10 and FRα were frequently expressed in TNBC and in ER positive and PR negative breast cancers. However, there may be different mechanisms by which SOX10 and FRα are implicated in aggressive breast cancers. These findings may help achieve a better understanding of these two different pathways involving potential stem cell maintenance (SOX10) and growth factors (FRα), their potential prognosis, and their therapeutic management of aggressive breast cancers in the future.

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800.799.9499
4040 Pike Lane
Concord CA 94520

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