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Sox2: a possible driver of the basal-like phenotype in sporadic breast cancer

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Tumours arising in *BRCA1* mutation carriers and sporadic basal-like breast carcinomas have similar phenotypic, immunohistochemical and clinical characteristics. *SOX2* is an embryonic transcription factor located at chromosome 3q, a region frequently gained in sporadic basal-like and *BRCA1* germline mutated tumours. The aim of the study was to establish whether sox2 expression was related to basal-like sporadic breast tumours. Two hundred and twenty-six sporadic node-negative invasive breast carcinomas were immunohistochemically analysed for oestrogen receptor (ER), progesterone receptor (PR), CK5/6, EGFR, vimentin, HER2, ki67, p53 and sox2 using tissue microarrays. Tumours were considered to have basal-like phenotype if they were ER/HER2-negative and CK5/6 and/or EGFR-positive. Thirty cases of this series (13.7%) displayed a basal-like phenotype. Sox2 expression was observed in 16.7% of cases and was significantly more frequently expressed in basal-like breast carcinomas (43.3% in basal-like, 10.6% in luminal and 13.3% in HER2+ tumours, $P<0.001$). Moreover, Sox2 showed a statistically significant inverse association with ER and PR ($P=0.001$ and 0.017, respectively) and direct association with CK5/6, EGFR and vimentin ($P=0.022$, 0.005 and <0.001, respectively). Sox2 is preferentially expressed in tumours with basal-like phenotype and may play a role in defining their less differentiated/'stem cell' phenotypic characteristics.
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Breast cancer is a heterogeneous disease encompassing a wide variety of pathological entities and a range of clinical behaviour.[1] Recent cDNA microarray studies have demonstrated that breast cancers can be classified according to their gene expression profiles into four main groups: basal-like, luminal (A and B), HER2+ and normal breast-like carcinomas.[1] Importantly, these groups have been shown to be of prognostic and predictive significance. Basal-like breast carcinomas are of high grade, lack oestrogen receptor (ER) and HER2 expression, and are consistently positive for basal keratins and/or other markers normally expressed by basal/myoepithelial cells.[1–9] Moreover, they characteristically overexpress cyclin E and show p53 immunoexpression. Historically, this phenotype was associated to breast carcinomas with a characteristic pattern of growth (consisting of a central area of necrosis and/or fibroelastosis surrounded by a ribbon of neoplastic growing at the periphery), and showing a poor prognosis and a peculiar proclivity for visceral metastasis, mainly to the lung and brain.[2,4,10–14] Recently, several studies[9,15–20] have demonstrated that basal-like carcinomas are characterised at the histological level by the presence of central scar, tumour necrosis, high proliferation rates, metaplastic elements (ie, spindle and squamous cells) and atypical medullary features. Therefore, it is not surprising that both metaplastic and typical/atypical medullary carcinomas have also been shown to belong to this subgroup of tumours.[19,20]

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The similarities between basal-like breast carcinomas and tumours arising in *BRCA1* germline mutation carriers are overwhelming.[21–24] *BRCA1* tumours clustered together with sporadic basal-like tumours in cDNA microarrays studies.[3] Moreover, these tumours also share immunohistochemical, morphological and biological characteristics, including the predisposition to visceral metastasis and better response to chemotherapy than other subgroup of tumours.[25,26] On the basis of these lines of evidence, many authors have suggested that *BRCA1* gene and/or BRCA1 pathway[27] are inactivated in basal-like sporadic tumours, which has been confirmed recently.[28]

SOX2 gene, located in chromosome 3q26.33, encodes for a member of the HMG-domain DNA-binding protein family. It has been demonstrated recently that sox2 may control the expression of several genes that play pivotal roles in embryonic development, including *nanog homeobox*, *nestin*, *δ-crystalline*, *fibroblast growth factor 4*, *undifferentiated embryonal cell transcription factor 1* and *F-box containing protein 15*. Moreover, its function is highly related to the expression of the *POU domain class 5 transcription factor 1* (aka, *OCT-4*), another gene implicated in maintaining stem cell properties. *SOX2* downregulation correlates with loss of pluripotency and self-renewal, and the activation of subsequent differentiation steps.[29–36] Furthermore, *SOX2* has been implicated as a target of *WNT, TGFB, FGF, BMP and NODAL* signalling pathways during embryogenesis.[37–41] *SOX2* gene expression has not been studied in breast cancer; however, gains of genomic material on the long arm of chromosome 3 (3q) are frequently found in tumours arising in *BRCA1* germ-line mutation carriers[42,43] and in sporadic basal-like breast cancer.[44]

Although there is no internationally agreement about the immunohistochemical definition of sporadic basal-like breast carcinomas, Nielsen *et al*[6] have demonstrated that a panel of four antibodies (ER, HER2, CK5/6 and EGFR) identifies these tumours with high specificity. Given the role played by sox2 in the maintenance of stem cells and the fact that basal-like cancers express multiple lineage markers (ie, luminal and basal keratins, vimentin, myoid markers) and frequently display metaplastic elements, we hypothesised that sox2 would be preferentially expressed in this subgroup of breast carcinomas. The aim of this study was to determine whether sox2 protein expression correlates with basal-like phenotype, according to Nielsen *et al.* criteria,[6] in a cohort of 226 node-negative invasive breast carcinomas.

Materials and methods

Tumour Samples

We analysed a group of 226 node-negative sporadic breast carcinomas diagnosed in Hospital Universitario La Paz, Madrid between 1988 and 2002. None of the patients had family history suggestive of familial/hereditary cancer.[45] Some of the clinicopathological characteristics of this series, including age, treatment, survival of the patients, as well as size, grade, histological subtype and some immunohistochemical characteristics of the tumours (oestrogen (ER) and progesterone receptors (PR), ki67, p53, HER2, CK5/6, EGFR and vimentin) have been reported previously.[14,46] The study was carried out with the approval of the 'Hospital La Paz' ethical committee.

Tissue Microarray Construction

Representative areas from formalin-fixed, paraffin-embedded infiltrating carcinomas and 30 samples from non-neoplastic breast tissue were carefully selected on H&E-stained sections and two 1-mm-diameter tissue cores were obtained from each specimen. The tissue cores were precisely arrayed into a new paraffin block using a tissue microarray workstation (Beecher Instruments, Silver Spring, MD).

Immunohistochemistry

Immunohistochemical staining on tissue microarray (TMAs) sections was performed using the EnVision method as described previously. Antibodies, clones, dilutions, antigen-retrieval methods and scoring systems for ER, PR, HER2, CK5/6, EGFR, vimentin, ki67 and p53 are described elsewhere.[14,46] Rabbit anti-sox2 polyclonal antibody was applied at dilution of 1:100 (sox2, Stem-cell Technologies). After a preheating step for antigen retrieval, TMA sections were immersed in boiling 10 mmol/l sodium citrate at pH 6.5 for 2 min in a pressure cooker. Cases were considered positive for sox2 when any unequivocal neoplastic cell displayed definite nuclear staining. Normal breast control cores on the TMA were used as internal controls. Positive (ie, normal breast tissue) and negative controls (ie, omission of the primary antibody and/or IgG-matched serum) were included in each slide run.

Tumours were subdivided according to the Nielsen *et al* classification as: basal-like (ER and HER2-negative and CK5/6 and/or EGFR-positive), luminal (ER-positive and HER2-negative) and HER2-positive subgroups.[6]

Statistical Analysis

To test associations between categorical variables we used the χ^2 or Fisher's exact test. Values of $P<0.05$ were considered significant. All tests were two-tailed and 95% confidence intervals were adopted. These analyses were carried out using the SPSS 12.0 for Windows statistical program (SPSS Inc., Chicago, IL).

Results

The correlations between sox2 expression, clinicopathological features and immunohistochemical markers studied in this series are summarised in Table 1.

Using the criteria proposed by Nielsen *et al*,[6] 30 cases (13.7%), 17 (7.8%) and 156 (71.2%) were classified as basal-like, HER2 and luminal cancers, respectively. Sixteen cases (7.3%) did not fulfil any of the above criteria and were excluded from the analysis. Sox2 nuclear expression was found in 33/198 (16.7%) node-negative breast carcinomas. Basal-like breast carcinomas showed a statistically significant higher prevalence of sox2 expression when compared to that seen in the other groups (Table 1, Figure 1): 13/30 basal-like breast carcinomas (43.3%) displayed sox2 immunoreactivity, whereas sox2 expression was seen in only 13.3% (2/15) of HER2 tumours and in 10.6% (14/132) of luminal tumours cancers ($P<0.0001$).

Sox2 expression was directly correlated with tumour size and expression of CK5/6, EGFR and vimentin, and inversely correlated with ER and PR expression.

Taken together, these findings demonstrate that sox2 is preferentially expressed in basal-like carcinomas. However, in a way akin to other proteins involved in the biology of basal-like carcinomas (eg, caveolin 1,[47] 14-3-3σ[48]), sox2 expression is not restricted to basal-like cancers, given that up to 10% of ER-positive tumours and up to 13% of HER2+ showed positivity for sox2.

Discussion

It has been demonstrated that tumours arising in *BRCA1* germline mutation carriers show significant histological, immunohistochemical expression profile and molecular genetic differences when compared to sporadic breast carcinomas. However, with the more comprehensive characterisation of basal-like breast carcinomas, it has become clear that tumours arising in *BRCA1* germline mutation carriers and sporadic basal-like breast carcinomas have genotypic and phenotypic traits that are remarkably similar.[2,3,7,8,10,11,14,16–20,22,23,27,28,49–58]

Comparative genomic hybridisation (CGH) analysis has demonstrated that 3q copy number gains were an independent predictor of poor prognosis in a cohort of 76 sporadic node-negative breast tumours[59] and that these gains are significantly more prevalent in tumours arising in *BRCA1* mutation carriers.[42,43] In addition, gains of the telomeric region of 3q are seen in approximately 20% of basal-like cancers and 10% of luminal tumours.[44]

Sox2, a transcription factor located on 3q26.3-q27, is one of the transcription factors expressed by stem cells. There is growing evidence to suggest that this gene is essential for the maintenance of stem cell proliferation and differentiation capabilities, and it is not expressed in mature differentiated cells.[29–41,60] Its role in carcinogenesis is poorly understood, although deregulation of homeobox gene expression has been implicated in the determining phenotypic expression of gastric, ampullar and pancreatic neoplastic cells and in the biology of cancers arising in these anatomical sites.[61–67] Furthermore, expression analysis studies have shown that *SOX2* is one of the genes differentially expressed between embryonal carcinomas and seminomas. Previous studies[39] have suggested that neoplastic cells of seminomas have phenotypic characteristics similar to those of undifferentiated germ cells, whereas embryonal carcinoma cells resemble pluripotent embryonic stem cells, which have the ability to differentiate.[39] Moreover, *SOX2* has been shown recently to be amplified in prostate cancers.[68]

In our series, sox2 nuclear expression was strongly associated with basal-like phenotype ($P<0.001$). Bertucci *et al*[69] have demonstrated a highly significant overrepresentation of genes located on 12p13 and 6p21.3 in basal-like tumours, including several genes related to stem cell biology, such as *NANOG*, *GDF3*, *STELLA*, *DPPA3*, *CD9* and *EDR* and *OCT4/POU5F1,* respectively. 12p13 cytogenetic band is reported to be a hots-pot region for structural chromosomal changes associated with germ cell tumours.[39] Although previous chromosomal CGH studies did not reveal gains of genomic material on 12p13 and/or 6p21.3 as a frequent event in basal-like and BRCA1 tumours,[55,70] more recent array CGH and fluorescent *in situ* hybridisation analysis have demonstrated that gains of genomic material on 6p21 and amplification of 12p13 are preferentially found in basal-like breast cancers.[44,71] These discrepancies may be because of the low resolution of chromosomal CGH and to the different definitions for basal-like cancers employed in these studies.[44,55,70,71] Interestingly, some of stem cell-related genes mapping to 12p13, are reported to be upregulated by *SOX2* gene expression and, on the other hand, sox2 is directly regulated by *OCT4*,[31,33,35,36,40] which maps to 6p21. Taken together, these findings suggest that in both sporadic basal-like carcinomas and tumours arising in *BRCA1* germline mutation carriers, sox2 expression may be either driven by *SOX2* gene copy number gains or *OCT4* gene-mediated *SOX2* transcriptional activation. Therefore, it seems clear that further studies are required to clarify the mechanism of sox2 expression in basal-like breast cancers.

Sox2 expression in our series was strongly correlated to CK5/6, EGFR and vimentin immunoreactivity. Both CK5/6 and EGFR proteins have been reported to be expressed by both normal mammary myoepithelial and epithelial cells[5,72,73] and, when included in a immunohistochemical panel together with ER and HER2, these proteins define with high specificity the basal-like phenotype in both sporadic and in tumour arising in *BRCA1* germline mutation carriers.[6,8,21,51–53] Vimentin is highly characteristic of

Table 1 Correlations between sox2 expression and clinicopathological and immunohistochemical features in node-negative breast carcinomas

	Sox2 negative (n = *165*)	*Sox2 positive* (n = *33*)	P
Menopausal status	70/84 (83.3%)	14/84 (16.7%)	
Premenopausal	88/106 (83.0%)	18/106 (17.0%)	1
Postmenopausal			
Size			
p T1	86/98 (87.8%)	12/98 (12.2%)	
p T2	49/67 (73.1%)	18/67 (26.9%)	0.023
Histological grade			
Grade 1	43/45 (95.6%)	2/45 (4.4%)	
Grade 2	40/49 (81.6%)	9/49 (18.4%)	
Grade 3	65/81 (80.2%)	16/81 (19.8%)	0.059
Clinical phenotype[a]			
Basal-like	17/30 (56.7%)	13/30 (43.3%)	
Luminal	118/132 (89.4%)	14/132 (10.6%)	
HER2+	13/15 (86.7%)	2/15 (13.3%)	<0.001
Oestrogen receptor			
Negative	41/59 (69.5%)	18/59 (30.5%)	
Positive	123/138 (89.1%)	15/138 (10.9%)	0.001
Progesterone receptor			
Negative	52/70 (74.3%)	18/70 (25.7%)	
Positive	111/126 (88.1%)	15/126 (11.9%)	0.017
HER2			
Negative	151/182 (83.0%)	31/182 (17.0%)	
Positive	14/16 (87.5%)	2/16 (12.5%)	0.1
p53			
Negative	118/139 (84.6%)	21/139 (15.1%)	
Positive	45/57 (78.9%)	12/57 (21.1%)	0.4
Ki67			
Negative	132/156 (84.6%)	24/156 (15.4%)	
Positive	33/42 (78.6%)	9/42 (21.4%)	0.356
EGFR			
Negative	149/173 (86.1%)	24/173 (13.9%)	
Positive	11/19 (57.9%)	8/19 (42.1%)	0.005
CK5/6			
Negative	140/162 (86.4%)	22/162 (13.6%)	
Positive	24/35 (68.6%)	11/35 (31.4%)	0.022
Vimentin			
Negative	132/148 (89.2%)	16/148 (10.8%)	
Positive	32/49 (65.3%)	17/49 (34.7%)	<0.001

CK5/6: cytokeratin 5/6; EGFR: epidermal growth factor receptor.
[a]According to the Nielsen *et al* criteria[6].

all immature primitive cells, and in normal breast its expression is restricted to mesenchymal and myoepithelial cells.[72,73,74] Its expression in breast tumours has been also related to a basal-like phenotype in both sporadic and familial tumours.[9,46,74,75] Several explanations have been put forward for the existence of breast cancers expressing both myoepithelial/mesenchymal and luminal markers: some have suggested that these lesions have a 'stem cell phenotype' and therefore would be able to be different towards different lineages, whereas others have speculated that this may reflect the ability of these cells to undergo epithelial–mesenchymal transition or to activate a basal/myoepithelial differentiation programme.[5,8,19,54,57,58,76]

Given the previously reported functions of SOX2, our results suggest that sox2 expression may play a role in conferring a less differentiated phenotype in these tumours or to activate the ability to differentiate in both luminal and basal/myoepithelial lineages.

In conclusion, our findings suggest that sox2 expression, a stem cell transcription factor, located in a frequently gained genomic region of *BRCA1*

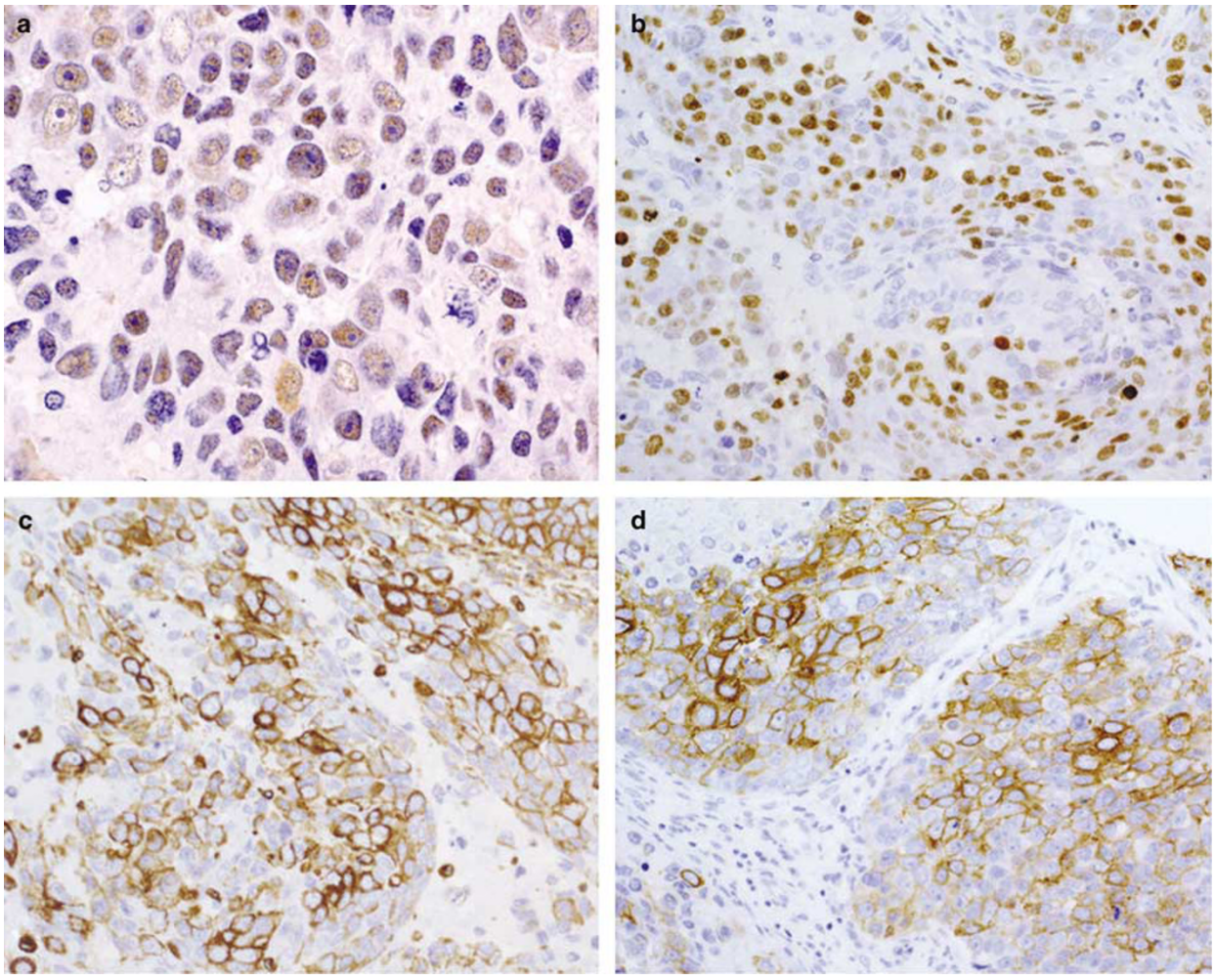

Figure 1 Expression of proteins studied by immunohistochemistry on tissue microarrays. Expression of sox2 (**a**), ki67 (**b**), vimentin (**c**) and CK5/6 (**d**), in one case of sporadic, lymph node negative, grade 3 invasive ductal carcinoma with basal-like phenotype. Original magnification: (**a**) ×400; and (**b–d**) ×200.

familial cancers and sporadic basal-like cancers, may play a role in the biology of basal-like breast carcinomas.

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