

Differential expression of testin and survivin in breast cancer subtypes

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Abstract. Testin (TES) is a putative tumour-suppressor gene downregulated in various types of cancers. Survivin is a nodal protein involved in multiple signalling pathways, tumour maintenance and inhibition of apoptosis. Previous studies indicate that TES and survivin can functionally interact and modulate cell death and proliferation in breast cancer cells. The aim of the present study was to investigate the expression and prognostic relevance of TES and survivin in breast cancer subtypes examining a large cohort of breast cancer patients. We determined the expression of TES and survivin by immunohistochemistry (IHC) in tissue samples from 242 breast cancer patients diagnosed between 1981 and 2009. The expression of these proteins was compared with clinical and pathological data. There was a significant association of nuclear survivin overexpression and TES downregulation with triple-negative tumours [P=0.009; univariate odds ratio (OR), 3.20; 95% CI, 1.34-7.66] (P=0.018; multivariate OR, 2.90; 95% CI, 1.20-6.97). A further significant correlation was observed between TES downregulation and the luminal B subtype (P=0.019, univariate OR: 2.90; 95% CI, 1.19-7.06) (P=0.032, multivariate OR, 2.67; 95% CI, 1.09-6.65), independent of survivin expression. Our results demonstrated a statistically significant association between TES downregulation and highly aggressive breast tumour subtypes, such as triple-negative and luminal B tumours, along with the prognostic relevance of nuclear expression of survivin. To our knowledge, this is the first demonstration of such an association.

Introduction

Breast cancer is the most common cancer among women worldwide and is a highly heterogeneous disease. Therefore, there is a pressing need for methods with which to stratify

patients into the different risk groups more accurately than the current clinicopathological classifications (1-4). A molecular-based approach to classify breast tumours was first proposed by Sørlie *et al* (5). In this study, breast carcinomas were clustered based on gene expression profiles determined using DNA microarrays. Breast tumours were divided into luminal A [estrogen receptor-positive (ER⁺) and/or progesterone receptor-positive (PR⁺)/human epidermal growth factor receptor 2-negative (HER2⁻)], luminal B (ER⁺ and/or PR⁺/HER2⁺), basal-like, HER2⁺ and normal-like breast cancer. These subtypes are associated with distinct prognosis and treatment options. Immunohistochemical-based molecular classifications have also been proposed alternatively (6-8). Immunohistochemistry (IHC) has been demonstrated to be an efficient and acceptable surrogate of gene expression analysis (6,9-11). Several lines of evidence have confirmed that the subclassification of ER⁺ cancers and the prognostic value of gene signatures is largely driven by the expression levels of proliferation-related genes and that proliferation markers, such as Ki67, may provide equivalent prognostic information to that provided by gene signatures. In particular, according to the new St. Gallen consensus recommendations, Ki67 is one of the prognostic markers that is considered important to subclassify luminal A and luminal B, together with HER2 expression (12). The absence of ER, PR and HER2 is used to define the triple-negative subtype, which represents ~15% of breast tumours and is not a homogeneous entity (13-15). Thus, new prognostic and/or predictive factors may provide additional risk stratifications to better guide treatment decisions in these different subtypes of breast cancer.

Survivin (also called baculoviral inhibitor of apoptosis repeat-containing 5, BIRC5) is a member of the family of inhibitor of apoptosis proteins (IAP) and is a multifunctional protein implicated in a number of cellular processes, including apoptosis, mitosis and angiogenesis (16). Survivin is present during fetal development and is rarely detectable, but is sometimes present in terminally differentiated normal adult tissues (17-19). Importantly, survivin is abundantly expressed in most types of cancers, including breast, colorectal, lung, gastric, bladder and liver cancer, melanoma and malignant lymphoma (20). The incidence of survivin expression in cancer is reported to range from 30 up to 100% (21). High survivin expression is associated with poor prognosis in most human

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Table I. Clinicopathological data of the patient cohort.

Variables	Total (n=242) (%)
Age (years, mean \pm SD)	54.6 \pm 12.0
Histologic subtype	
Ductal	219 (90.5)
Lobular	23 (9.5)
Lymph node status	
Negative	134 (55.4)
Positive	104 (42.9)
Unknown	4 (1.7)
Histologic grade	
I	32 (13.2)
II	122 (50.4)
III	87 (36.0)
Unknown	1 (0.4)
Tumour stage	
0	7 (2.9)
I	92 (38.0)
II	111 (45.9)
III	28 (11.6)
Unknown	4 (1.7)
ER	
<10%	22 (9.1)
\geq 10%	220 (90.9)
PR	
<10%	85 (35.1)
\geq 10%	157 (64.9)
Ki67	
<10%	66 (27.3)
\geq 10%	172 (71.1)
Unknown	4 (1.7)
HER2 IHC	
Negative/moderate (0-2+)	208 (85.9)
Strong (3+)	27 (11.2)
Unknown	7 (2.9)
Event	
NED	197 (81.4)
Local recurrence	10 (4.1)
Metastasis	15 (6.2)
Contralateral tumour	12 (5.0)
Death	8 (3.3)

ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

cancers. Although it exhibits a high degree of tumour-specific expression and is one of the 16 cancer-related genes included in the Oncotype DX assay (22), the role of survivin as a breast cancer biomarker has remained the subject of much debate. Previous studies using quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and IHC have reported

that survivin is either prognostically irrelevant or associated with improved or adverse outcome in primary breast cancer patients (23-25). Such discordant results could perhaps be explained by the fact that these studies did not account for subcellular localization of survivin, which can be present in both nuclear and cytoplasmic pools. These different pools are immunochemically and functionally different and are independently modulated during the cell cycle (26). Furthermore, recent studies have demonstrated that increased expression of nuclear, as opposed to cytoplasmic, survivin was associated with decreased overall survival (OS) and breast cancer-specific survival (BCSS) (27,28).

Testin (TES) is a putative tumour suppressor. The human TES gene is localised to the fragile site FRA7G at 7q31.2, and downregulation of TES has been reported in many human malignancies (29-33). In addition, a profound reduction in growth potential was detected in different cancer cell lines in which TES was overexpressed (34,35). TES is a highly conserved protein of 421 amino acids containing three C-terminal LIM domains, which are responsible for protein-protein interactions coordinating intracellular and extracellular pathways. In particular, TES is a component of the focal adhesion complex, which is important in the regulation of epithelial physiology and localises to cell-matrix adhesions, cell-cell contacts and actin stress fibres. In mice, TES interacts and colocalises with a variety of cytoskeletal proteins, including zyxin, mena, VASP, talin and actin (36,37). Overexpression of TES decreased cell motility (36-38). Moreover, restoration of TES expression in breast cancer and uterine sarcoma cell lines inhibited their growth by induction of apoptosis (34). In association with alterations of cell adhesion and motility, TES expression resulted in activation of caspase-dependent and -independent apoptosis in the absence or with a reduced level of survivin (34).

Expression of TES and its relationship with survivin have never been evaluated in a large series of human breast tumours. The aim of this study was to determine whether TES and survivin expression could characterise the different breast cancer subtypes and their correlation with clinicopathological parameters.

Materials and methods

Patient samples. The study was carried out on 242 consecutive cases of breast carcinomas that were obtained from the Cantonal Institute of Pathology (Locarno, Switzerland). The study was approved by the Cantone Ticino Ethics Committee. All cases were diagnosed during the period from January 1981 to December 2009 with a median follow-up time of 5.2 years (SD, 3.4 years). The median age at diagnosis was 54.4 (SD, 12.0). The histological diagnosis was determined during routine pathological assessment. The tumours were graded according to the Scarff-Bloom Richardson classification as modified by Elston and Ellis (39). Staging at the time of diagnosis was based on the TNM system (40). The clinicopathological characteristics of the patients are listed in Table I. All patients underwent surgery \pm radiotherapy and systemic standard treatment. Survival data, including disease-free survival (DFS) and BCSS, were maintained on a prospective basis. DFS was defined as the interval (in months) from the

date of the primary surgical treatment to the first loco-regional or distant recurrence and BCSS was defined as the time (in months) from the date of the primary surgical treatment to the time of death from breast cancer.

Tumour classification. Tumours were classified according to standard molecular subtypes as follows: luminal A type (154/242, 63.6%), luminal B type (39/242, 16.1%; in particular 24/242, 9.9%, with both ER and PR positivity), HER2 overexpression type (7/242, 2.9%), and triple-negative type (35/242, 14.5%).

Immunohistochemistry. Sections (3- μ m) were cut from formalin-fixed paraffin-embedded (FFPE) blocks and mounted on positive-charged slides. Immunostaining was performed using anti-survivin rabbit polyclonal antibody (1:250, cat. ab469) and anti-TES mouse monoclonal antibody (1:200, cat. ab57292; both from Abcam, Cambridge, MA, USA). The specificity of both antibodies was previously confirmed by western blot analysis. Cell nuclei were counterstained with hematoxylin solution. Slides were evaluated by at least two investigators in a blinded manner. Positive samples for each antibody and negative samples, in which the primary antibody was omitted, were used as controls. Adjacent normal breast tissues in most samples served as the internal negative or positive control depending on the protein tested.

Data analysis. Immunostaining for survivin was recorded according to staining intensity, distribution in the cytoplasm and/or nucleus and the percentage of positive tumour cells. In cases where staining was heterogeneous in the slide, examined fields included those with the highest and lowest percentage of stained cells. A mean percentage of positive tumour cells was determined in at least five areas at a magnification of x400. A tumour was assessed as survivin-positive if the staining was positive-cytoplasmatic, positive-nuclear or both. Staining was scored as follows: score 0, no staining or staining in <5% of cells; score 1, weak staining in 6-19% of cells; score 2, moderate staining in 20-40% of cells; score 3, strong staining in >40% of cells. For statistical analysis, scores 0 and 1 were considered negative, and scores 2 and 3 were considered positive for both cytoplasmatic and nuclear staining. Immunostaining for cytoplasmatic TES was scored as follows: score 0, no staining or staining in 2% of cells; score 1, 3-40% positively stained cells (weak staining); score 2, 41-65% positively stained cells (moderate staining); score 3, >65% positively stained cells (strong staining).

Data on ER, PR, Ki67 and HER2/neu were obtained through standard clinical testing, using IHC for ER and PR and the Hercep Test™ (Dako, Glostrup, Denmark) for HER2/neu. HER2 staining was divided into two groups, with negative to moderate (0-2+) HER2 expression and strong (3+) overexpression. Cases scoring 2+ for HER2 immunostaining were subsequently assessed by fluorescence *in situ* hybridisation study (FISH).

Statistical analysis. The statistical analysis was carried out using the semi-quantitative results of the immunohistochemical staining. A univariate statistical analysis was carried out using Chi-Square test and odds ratios (ORs) to

analyse the categorical variables and the Student's t-test for independent samples to analyse continuous variables. The relationship between 'survivin overexpression and TES down-regulation' with 'triple-negative phenotype' was also studied in the multivariate analysis, taking into account the effect of the confounding variable 'histological grade', using a binary logistic model. Continuous data were tested for normality. The analysis of time to event was performed using Kaplan-Meier methodology. Statistical significance was defined as a value of $P=0.05$, two-tailed. All statistical analyses were performed using PASW Statistics 19 (formerly SPSS 19).

Results

Expression of survivin and TES in breast tissues. Cytoplasmatic or nuclear survivin expression was detected in 97% of the breast carcinomas. No expression of survivin was observed in the adjacent normal breast tissues, with the exception of a few samples where the normal breast tissues showed cytoplasmic positivity. The above percentage was within the range of previously published studies (17,18,41). Positive nuclear staining of survivin was found in 9/242 of the breast tumours (3.7%; score 2 and 3), while positive cytoplasmic staining was present in 22.3% of the cases (54/242). In 172 out of 242 (71%) patients there was positive staining both in the nucleus and in the cytoplasm (Fig. 1). These results are in accordance with previous studies (42).

Reduced TES expression was found in 74.7% of the cases (181/242). Tumours were divided into three different categories: no staining, weak staining and moderate staining (score 0, 1 and 2, respectively) (Fig. 2). The adjacent normal breast tissues with surrounding mesenchymal and endothelial cells showed specific immunoreactivity and represented an internal positive control for TES antibody specificity.

Correlation between survivin and TES expression and clinical outcome of breast cancer patients. From a clinical perspective, we assessed the correlation between survivin and TES expression and clinicopathological parameters in the primary breast cancers (Table II). TES status, alone and in association with nuclear expression of survivin, was significantly correlated with increased histological grade, being predominantly present in grade III tumours ($P=0.004$ and 0.003 , respectively). There was also a statistically significant correlation between high nuclear survivin expression (>40% of tumour cells) and the presence of lymph node metastases ($P=0.09$). There was no significant association between nuclear and/or cytoplasmic survivin expression, TES expression and age of patients, histological type (ductal or lobular) and HER2 status. However, there was a trend toward a significant association between absent or weak expression of TES and Ki67 expression ($P=0.055$). The results of the χ^2 analysis of these data are summarised in Table II.

On account of the short median follow-up time of 5.2 years, Kaplan-Meier survival analysis did not reveal any association between survivin and TES expression and BCSS or DFS. However, we found a higher percentage of events (i.e. local recurrence, distant metastases and death) in cases with positive nuclear survivin expression (22.2 vs. 11.3%, $P=0.039$) and low TES expression (23.8 vs. 14.6%, $P=0.068$) (Tables I and III).

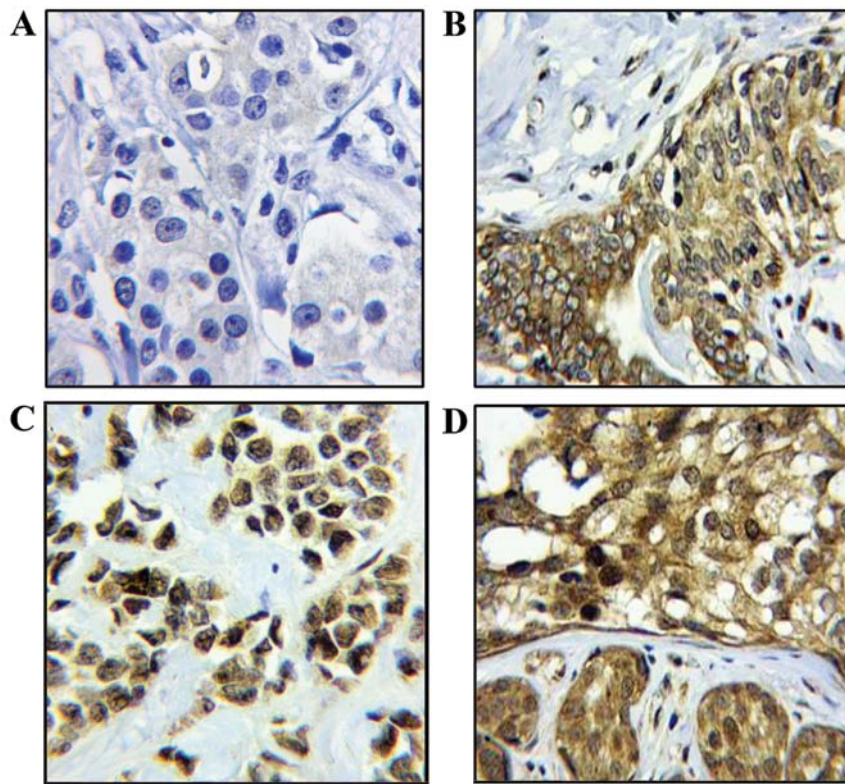


Figure 1. Representative images of the results of survivin IHC (magnification, x400). (A) Negative cytoplasmic and nuclear expression of survivin. (B) Positive cytoplasmic expression of survivin. (C) Positive nuclear expression of survivin. (D) Positive cytoplasmic and nuclear expression of survivin.

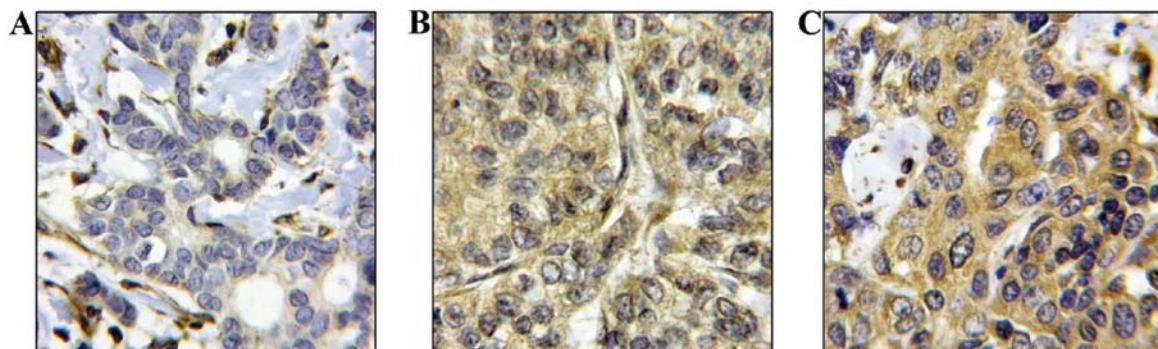


Figure 2. Representative images of TES IHC (magnification, x400). (A) Negative cytoplasmic expression of TES. (B) Weak cytoplasmic expression of TES. (C) Moderate cytoplasmic expression of TES.

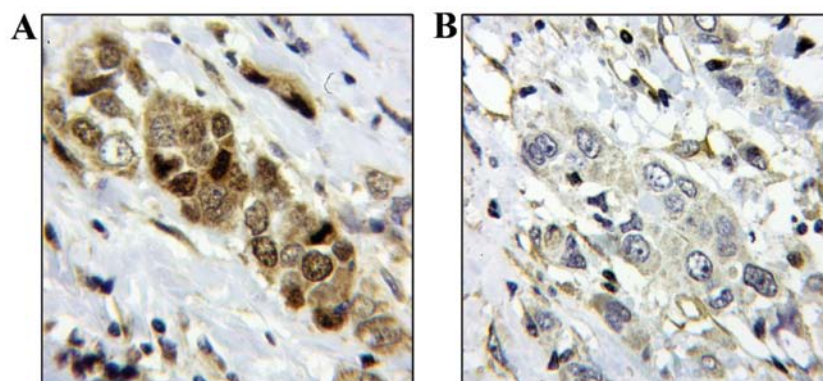


Figure 3. Triple-negative subtype staining. (A) Triple-negative breast cancer with nuclear and cytoplasmic expression of survivin and (B) negative expression of TES (magnification, x400).

Table II. Relationship between survivin and TES expression and standard clinicopathological and immunohistochemical markers.

Parameter	Nuclear survivin high expression (score 2-3) n (%)	Chi-square P-value	TES negative/low/ reduced expression (score 0-2) n (%)	Chi-square P-value	Association of survivin (score 2-3) and TES (score 0-2) n (%)	Chi-square P-value
Grade						
1	15/32 (46.9)	0.097 NS	19/32 (59.4)	0.004	13/32 (40.6)	0.003
2	60/122 (49.2)		87/122 (71.3)		67/122 (54.9)	
3	45/87 (51.7)		75/87 (86.2)		63/87 (72.4)	
Histological nodal status						
Negative	57/134 (42.5)	0.009	54/134 (40.3)	0.224 NS	78/134 (58.2)	0.814 NS
Positive	62/104 (59.6)		51/104 (49)		63/104 (60.6)	
Ki67						
<10	45/66 (68.2)	0.146 NS	22/66 (33.3)	0.055 NS	33/66 (50)	0.087 NS
≥10	133/172 (77.3)		81/172 (47.1)		107/172 (62.2)	

TES, testin; NS, not significant.

Table III. Correlation of survivin and TES expression with event incidence.

	Survivin high expression (score 2-3) n (%)	Survivin negative or low expression (score 0-1) n (%)	Chi-square P-value	TES negative/ low expression (score 0-1) n (%)	TES moderate/ strong expression (score 2-3) n (%)	Chi-square P-value
Event incidence	36/162 (22.2)	9/80 (11.3)	0.039	25/105 (23.8)	20/137 (14.6)	0.068 NS
NED	126/162 (77.8)	71/80 (88.8)		80/105 (76.2)	117/137 (85.4)	

TES, testin.

Association of survivin and TES expression with breast cancer subtypes. The prognostic implications of breast cancer subtypes have been described in several reports. We found a statistically significant association between the subcellular localization of survivin (moderate/strong nuclear staining with or without cytoplasmic staining), reduced TES expression (score 0-2) and the triple-negative breast cancer subtype ($P=0.009$) (univariate OR, 3.20; 95% CI, 1.34-7.66) (Fig. 3). There was also a significant association between nuclear survivin expression and the triple-negative subtype ($P=0.022$) (univariate OR, 4.15; 95% CI, 1.22-14.1). A multivariate analysis based on a binary logistic model was carried out, using the variable 'survivin overexpression and TES downregulation' as the dependent one and 'histological grade' as well as 'triple-negative phenotype' as covariates. The histological grade was dichotomised into high (grade 2 and 3) and low (grade 1). Triple-negative phenotype exhibited a statistically significant result [$P=0.018$; OR, 2.90; 95% CI, 1.2-6.97 (reference group = no triple phenotype)], while the histological grade exhibited borderline significance [$P=0.051$; OR, 2.15; 95% CI, 0.997-4.62 (reference group = low histological grade)]. These results showed a

significant association between the triple-negative phenotype and survivin overexpression and TES downregulation, independently of the histological grade. Furthermore, there was a significant correlation between the absence or low expression of TES (immunohistochemical score 0-1) and the luminal B subtype with ER⁺ and PR⁺ expression ($P=0.019$) (univariate OR, 2.9; 95% CI, 1.19-7.06), independently of the histological grade (adjusted multivariate OR, 2.67; 95% CI, 1.09-6.65; $P=0.032$) (Fig. 4). These data are summarised in Table IV. Instead, there was no significant association between cytoplasmic and/or nuclear survivin expression, absence or downregulation of TES and the luminal A and HER2 subtypes.

Discussion

Advances in high-throughput methodologies have revolutionised the scientific approach to highly complex diseases. Breast cancer subtypes have been extensively characterised by gene expression analysis using microarrays. However, this approach is not feasible for large-scale clinical applications or retrospective studies using FFPE tissue samples. In

Table IV. Correlation between survivin and TES expression and breast cancer molecular subtypes.

Subtype n (%)	Nuclear survivin high expression (score 2-3) n (%)	Fisher test P-value	TES negative/low/ reduced expression (score 0-2) n (%)	Fisher test P-value	Association of survivin (score 2-3) and TES (score 0-2) n (%)	Fisher test P-value
Triple-negative 35/242 (14.89)	32/35 (91.4)	0.012	29/35 (82.9)	0.295 NS	28/35 (80.0)	0.009
	vs. 149/207 (72.0)		vs. 152/207 (73.4)		vs. 115/207 (55.6)	
	Univariate OR=4.15	0.022	Univariate OR=1.75	0.24 NS	Univariate OR=3.20	0.009
	95% CI, 1.22-14.1		95% CI, 0.69-4.44		95% CI, 1.34-7.66	
	Adjusted ^a OR=4.07	0.025	Adjusted ^a OR=1.54	0.37 NS	Adjusted ^a OR=2.90	0.018
	95% CI, 1.19-13.88		95% CI, 0.60-3.95		95% CI, 1.20-6.97	
Luminal B 24/242 (9.9)	17/24 (70.8)	0.63 NS	16/24 (66.6)	0.018	15/24 (62.5)	0.83 NS
	vs. 164/218 (75.2)		vs. 89/218 (40.8)		vs. 128/218 (58.7)	
	Univariate OR=0.80	0.64 NS	Univariate OR=2.90	0.019	Univariate OR=1.17	0.72 NS
	95% CI, 0.32-2.03		95% CI, 1.19-7.06		95% CI, 0.49-2.80	
	Adjusted ^a OR=0.77	0.59 NS	Adjusted ^a OR=2.67	0.032	Adjusted ^a OR=1.06	0.90 NS
	95% CI, 0.30-1.98		95% CI, 1.09-6.65		95% CI, 0.44-2.55	

^aOR adjusted for the effect of the confounding variable 'histological grade'. Luminal B, ER/PgR/HER2⁺. TES, testin; OR, odds ratio; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2. NS, not significant.

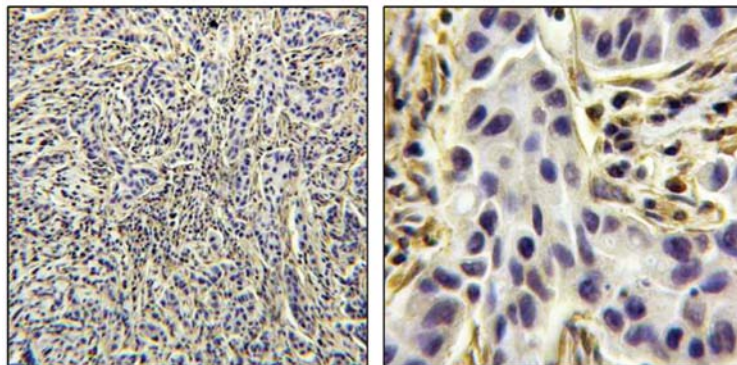


Figure 4. Luminal B subtype staining. Luminal B breast cancer (ER/PgR/HER2⁺) with negative expression of TES (magnification: x100, left panel; x400, right panel).

these situations immunohistochemical staining for specific biomarkers provides a useful alternative. In the present study, we showed that decreased expression of TES and increased levels of nuclear survivin were preferentially associated with the triple-negative subtype. This subtype generally presents high histological grade, Ki67 overexpression and unfavourable prognosis.

Survivin is a bifunctional protein, which is both an integral component of the chromosome passenger complex and a negative regulator of apoptosis. Survivin exists in distinct intracellular pools. The predominant cytosolic fraction and

a smaller nuclear pool are independently modulated during cell cycle progression and control the assembly of a normal bipolar mitotic apparatus (43). More importantly, cytoplasmic localisation of survivin in non-malignant cells suppresses apoptosis, while nuclear translocation may be important to regulate proliferation (44). Survivin intracellular localisation is regulated by an active and evolutionarily conserved, Crml-dependent nuclear export signal, which appears to be essential for survivin tumour-promoting functions. In particular, inhibition of this signal abrogates the anti-apoptotic effect of survivin, while maintains its mitotic activity. This suggests

that increased levels of nuclear survivin lead to a proliferative aggressive phenotype (28,45,46). However, the exact prognostic and clinical implications of the nuclear or cytoplasmatic localisation of survivin remain controversial. Here, we found that nuclear survivin is a predictor of worse outcome in breast cancer and a strong association between nuclear survivin and the triple-negative breast cancer subtype.

Based on the results of our previous study on TES in breast cancer cell lines (34), we assessed the pattern of TES expression in breast tumours to determine whether reduced expression of TES would be preferentially associated with specific tumour subgroups. We found that TES was expressed in ducts and lobules of normal breast. In particular, TES was present in epithelial, myoepithelial and basal cells in normal tissues. TES was significantly reduced in tumour cells in a large fraction of the breast cancers. In some breast tumours with adjacent normal tissues with hyperplastic foci, TES was detected only in the normal myoepithelial and basal cells. Negative expression of TES in the columnar cells is likely to be the result of dysplastic transformation of the breast epithelium. However, the significance of this particular localisation warrants investigation, as it suggests that the pattern of expression of TES is more complex than originally believed. TES was also abundantly expressed in the stroma and endothelial cells (47) surrounding both normal and tumour tissues. Remarkably, we found a significant correlation between TES downregulation, together with nuclear survivin expression, and the triple-negative subtype. In contrast, regardless of survivin, negative or very weak expression of TES was strongly correlated with the luminal B subtype, ER⁺ and PR⁺ tumours.

Tumourigenesis is a multistep process resulting from the accumulation of genetic and epigenetic changes (48,49). DNA methylation, a major epigenetic modification, leads to gene silencing. The frequency of hypermethylation of CpG dinucleotides varies significantly between breast cancer subtypes (50). CpG islands were found to be more frequently methylated in luminal B tumours than in the other tumour subtypes (50). Furthermore, depending on ER status and irrespective of the molecular subtype, a higher methylation frequency was observed in ER⁺ and PR⁺ tumours (50). The human TES gene is located in the fragile chromosomal region FRA7G. Common fragile sites are regions in mammalian chromosomes prone to breakage and rearrangements. This genetic instability can lead to disease manifestations and may play a role in oncogenesis (51). FRA7G is a locus of 300 kb, localised between markers D7S486 and D7S522, which shows loss of heterozygosity in many human malignancies (52,53). This region is known to encompass several genes, in addition to TES, including caveolin-1, caveolin-2 (54) and MET (55). The methylation of CpG in the TES promoter is a frequent event in gastric tumours (32). In previous studies, methylation of the TES promoter was also shown to be common in breast cancer (30,34) and may be involved in TES downregulation. The different correlation of TES in regards to triple-negative and luminal B subtypes could be linked to a different grade of hypermethylation of CpG islands in the TES promoter region (50).

TES is an important structural protein and may serve as a platform to integrate multiple signal transduction events. Current data suggest the possibility that downregulation of TES

is associated with alterations in cell adhesion and motility and therefore can lead to development of tumours with an aggressive phenotype. The reduced expression of TES in tumours of the basal-like/triple-negative subtype, along with its expression in myoepithelial/basal cells of the normal breast, can lead to speculate a possible role in epithelial-to-mesenchymal transition (EMT). EMT is an important process associated with the ability of epithelial cells to detach from a primary tumour and metastasise. It is also possible that the tumour-suppressive function of TES may reside within alternative, yet unknown functions. A possible function of TES in survivin-dependent pathways may stem from maintenance of a basal/myoepithelial phenotype in basal-like/triple-negative breast cancer, as it has been noted for caveolin 1 and 2 (56,57).

Reduced expression of TES characterises breast cancer subtypes with particularly poor outcome such as triple-negative and luminal B tumours, and therefore can be considered an important marker to aid in predicting the course of disease, either by itself or in association with established markers, such as survivin. Further studies generating long-term follow-up data are warranted to confirm the usefulness of TES as a biomarker in breast cancer. Furthermore, a greater understanding of the molecular and functional role of TES in aggressive types of breast cancer may lead to more selective and effective treatment for breast cancer patients.

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