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Loss of STARD10 expression identifies a group of poor prognosis breast cancers independent of HER2/Neu and triple negative status

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The phospholipid transfer protein STARD10 cooperates with *c-erbB* signaling and is overexpressed in *Neu/ErbB2* breast cancers. We investigated if STARD10 expression provides additional prognostic information to HER2/*neu* status in primary breast cancer. A published gene expression dataset was used to determine relationships between *STARD10* and *HER2* mRNA levels and patient outcome. The central findings were independently validated by immunohistochemistry in a retrospective cohort of 222 patients with breast cancer with a median follow-up of 64 months. Kaplan–Meier and Cox proportional hazards analyses were used for univariate and multivariate analyses. Patients with low *STARD10* or high *HER2* tumor mRNA levels formed discrete groups each associated with a poor disease-specific survival ($p = 0.0001$ and $p = 0.0058$, respectively). In the immunohistochemical study low/absent STARD10 expression *i.e.* $\leq$10% positive cells was observed in 24 of 222 (11%) tumors. In a univariate model, low/absent STARD10 expression was significantly associated with decreased patient survival ($p = 0.0008$). In multivariate analyses incorporating tumor size, tumor grade, lymph node status, ER, PR and *HER2* status, low STARD10 expression was an independent predictor of death from breast cancer (HR: 2.56 (95% CI: 1.27–5.18), $p = 0.0086$). Furthermore, low/absent STARD10 expression, *HER2* amplification and triple negative status were independent prognostic variables. Loss of STARD10 expression may provide an additional marker of poor outcome in breast cancer identifying a subgroup of patients with a particularly adverse prognosis, which is independent of HER2 amplification and the triple negative phenotype.

Key words: breast cancer, STARD10, HER2/Neu, triple negative breast cancer
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Breast cancer is a complex, heterogeneous disease, encompassing a range of pathologies and patterns of gene expression resulting in disparate clinical behavior and response to therapy.[1,2] Axillary lymph node status, tumor size and grade, patient age and lymphovascular invasion are among the clinicopathological prognostic indicators currently employed to predict an individual's risk of metastasis.[3] Axillary lymph node status is the best clinicopathological prognostic marker available, but it is an imperfect predictor. Approximately 25% of node-negative patients harbor micrometastases and are destined to relapse. Conversely, up to 50% of node-positive patients do not recur after many years of follow-up even without adjuvant treatment.[4] Thus, there is a critical need to identify more accurate prognostic markers that can identify a patient's risk of recurrence and to accurately select which adjuvant therapeutic regimens are most likely to benefit individual patients.

Currently, the only recommended predictive biomarkers in breast cancer are the estrogen (ER) and progesterone receptors (PR) for selecting endocrine-sensitive disease[3,5] and the human epidermal growth factor receptor 2, *HER2* (*c-erbB2/neu*), for identifying patients who may benefit from trastuzumab, a therapeutic antibody targeting the *c-erbB2* receptor.[5,6] *HER2* status also indicates an enhanced sensitivity to high dose anthracycline-based chemotherapeutic regimens,[7] while HER2 overexpression is associated with a lower probability of response to tamoxifen therapy in both early and advanced breast cancer.[8,9] Advances in expression microarray technology allow classification of intrinsic subtypes of breast cancer[2] which identify basal-like or triple negative phenotype breast cancers (*i.e.* ER, PR and HER2 negative, TNP) as poor prognosis groups.[10,11] Other gene "signatures" that also predict disease outcome include the 70 gene signature,[12] 21-gene "signature" (Oncotype DX® RT-PCR assay)[13] and the genomic grade index.[14] However, these advances have yet to be translated into widespread clinical practice. Thus, there remains a need for the identification of further single gene or multiple prognostic markers of breast cancer, which are amenable to clinical practice, either alone or in combination.

STARD10, a recently described member of the START (steroidogenic acute regulatory proteins) lipid transfer proteins,[15,16] is overexpressed in mammary tumors from *Neu/ErbB2* transgenic mice and in 35% of primary human breast cancers.[15] Furthermore, increased expression of STARD10 is correlated with *c-erbB2/HER2* status in human breast cancer cell lines and a series of breast carcinomas while coexpression with the epidermal growth factor receptor (EGFR/*c-erbB1*) in murine fibroblasts enhances anchorage-independent cell growth[15] Together, these data imply potential functional cooperation between STARD10 and *ErbB* receptor signaling in breast oncogenesis.

This study was undertaken to further define STARD10 expression in a large cohort of patients with breast cancer, identify any relationship with known prognostic markers and determine if STARD10 status could provide additional prognostic information to *HER2* status in primary breast cancer.

Material and Methods

Gene expression data and analysis

Published gene expression data[17] were downloaded from http://microarray-pubs.stanford.edu/wound_NKI/explore.html and employed to establish relationships between STARD10 and HER2 mRNA levels and breast cancer outcome. Criteria employed for the choice of this dataset included: the presence of probesets for *STARD10* on the oligonucleotide array, the size of the cohort ($n = 295$) and length of follow-up (median 7.8 years, range 0.05–18.3) and the ability of classical clinicopathological parameters (tumor size, grade and lymph node status), ER and HER2 to predict outcome.

Patient cohort

Following approval from the Human Ethics Committee of St. Vincent's Hospital, a total of 222 patients diagnosed with invasive ductal breast carcinoma (IDC) and treated by a single surgeon (PC) between February 1992 and August 2002 was included in this study. The clinicopathological variables, Cox univariate analyses, event rates and median time to event are recorded in Table 1. Formalin-fixed, paraffin embedded tissue was available from the pathology archives of St. Vincent's Public Hospital (Sydpath) and St. Vincent's Private Hospital (Douglas Hanly Moir), Sydney, Australia. All tumors were classified as IDC according to the World Health Organization (WHO) schema[18] and were graded using the Nottingham combined histologic grading scheme.[19] Follow-up intervals were calculated from the date of definitive procedure (biopsy/lumpectomy/mastectomy) to the date of last recorded follow-up (mean: 67 months, median: 64 months, range: 1–154 months). These data were obtained from annual review of patient charts or tumor registry data for patients alive at the previous annual review. Patients less than 50 years of age with node-positive, ER− tumors or tumors larger than 3 cm received adjuvant chemotherapy (cyclophosphamide, methotrexate and 5-fluorouracil or adriamycin and cyclophosphamide (AC)). Patients with ER+ tumors who were more than 50 years of age received 5 years of tamoxifen therapy. Tissue microarrays (TMAs) were constructed from formalin-fixed, paraffin embedded tumor material; each patient was represented by 2–6, 1 mm cores.

Immunohistochemistry

Four-micron sections were cut from each TMA, mounted on SuperFrost®Plus glass slides, dewaxed by passage through xylene (two 5-min washes), cleared and rehydrated in graded alcohol (100%, 95% and 70%) ending in a distilled water wash. Endogenous peroxidases and proteins were blocked with 3% hydrogen peroxide and protein block, serum free (DAKO Corporation Carpenteria, CA). Sections, without antigen retrieval, were incubated with a 1:100 dilution of an affinity purified rabbit anti-STARD10 antibody,[15] and detection was performed with Envision+Rabbit (DAKO) for 30 min at room

Table 1. Cox univariate analysis, event rates and survival

	Hazard ratio (95% CI)	*p* value	Event rate	Median time to event (months)
Univariate analysis				
Grade 3	4.250 (2.152–8.395)	<0.0001	34/103	33.2
Size > 20 mm	2.901 (1.562–5.417)	0.0008	30/97	33.9
Lymph node +	2.877 (1.509–5.486)	0.0013	32/111	35.4
ER+	0.282 (0.155–0.514)	<0.0001	20/152	49.3
PR+	0.171 (0.084–0.347)	<0.0001	10/119	62.7
HER2 IHC 3+	4.592 (1.767–6.288)	<0.0001	12/25	33.9
HER2 amp (FISH)	3.334 (1.767–6.288)	0.0002	16/43	35.7
Triple negative	2.941 (1.549–5.587)	0.001	14/34	35.7
Low StarD10	3.065 (1.549–6.066)	0.0013	11/24	20.3
Survival				
Mean follow-up		67 months (0.1–154)		
Alive		165 (74%)		
Death due to breast cancer		45 (20%)		
Death due to other causes		12 (6%)		
Disease specific survival				
5 years		189 (85%)		
10 years		175 (79%)		

ER, estrogen receptor; PR, progesterone receptor; FISH, fluorescent *in-situ* hybridization.

temperature followed by visualization using DAB+ (DAKO) for 5 min. Slides were also stained using the following monoclonal antibodies: ER 1:100 (6F11, DAKO), PR 1:200 (PgR-636, DAKO). HER2/*neu* immunohistochemistry was undertaken using standard procedures,[20] and *HER2* FISH was assessed in The Australian National HER-2 Reference laboratory (SydPath, St Vincent's Hospital Sydney). A *HER2*:chromosome 17 ratio >2.2 was classified as *HER2* amplification.

Immunohistochemical scoring

For STARD10, 2–6 individual cores were assessed independently by 3 pathologists (EKAM, SAO'T, MC) who were blinded to each others results and to patient outcome. Scores were assigned as a percentage of cells with positive cytoplasmic and/or nuclear staining and the absolute intensity of staining determined on a scale of 0–3 where 0 represents no staining, 1 represents mild staining, 2 represents moderate staining and 3 represents strong staining. Criteria to achieve a positive score for STARD10 expression were cytoplasmic staining intensity >1 in >10% of cells. Where scoring varied between individual pathologists, a final score was resolved by conferencing and consensus. Scoring of ER/PR/HER2/*neu* immunohistochemical staining was performed by a single breast pathologist (EKAM) according to routine clinical practice guidelines. Assessment of HER2/*neu* positive immunostaining was restricted to areas demonstrating a membranous staining pattern. HER2/*neu* was classified as negative (≤2+) *versus* positive (3+). Triple negative was defined as ER, PR and HER-2 negative.

Statistical analyses

Statistical analyses were performed using Statview 5.0 Software (Abacus Systems, Berkeley, CA). A *p*-value of <0.05 was accepted as statistically significant. Correlations between STARD10 expression and clinicopathological variables were tested by applying cross tables and the Exact Fisher's Test. Disease-specific survival, defined as date of definitive procedure to date of death due to breast cancer, was selected as the primary endpoint for survival analysis. Kaplan–Meier and the Cox proportional hazards model were used for univariate and multivariate analyses, respectively. Those factors that were prognostic on univariate analysis were then assessed in a multivariable model to identify factors that were independently prognostic and those that were the result of confounding.

Results

STARD10/HER2 mRNA levels and association with patient outcome

An initial characterization of *STARD10* gene expression in breast cancer and its potential relationship to patient outcome was undertaken by interrogating the data of Chang *et al.*[17] When modeled as a continuous variable, higher expression levels of STARD10 mRNA were associated with longer survival (HR 0.25, 95%CI: 0.09–0.64, $p = 0.004$). As loss of

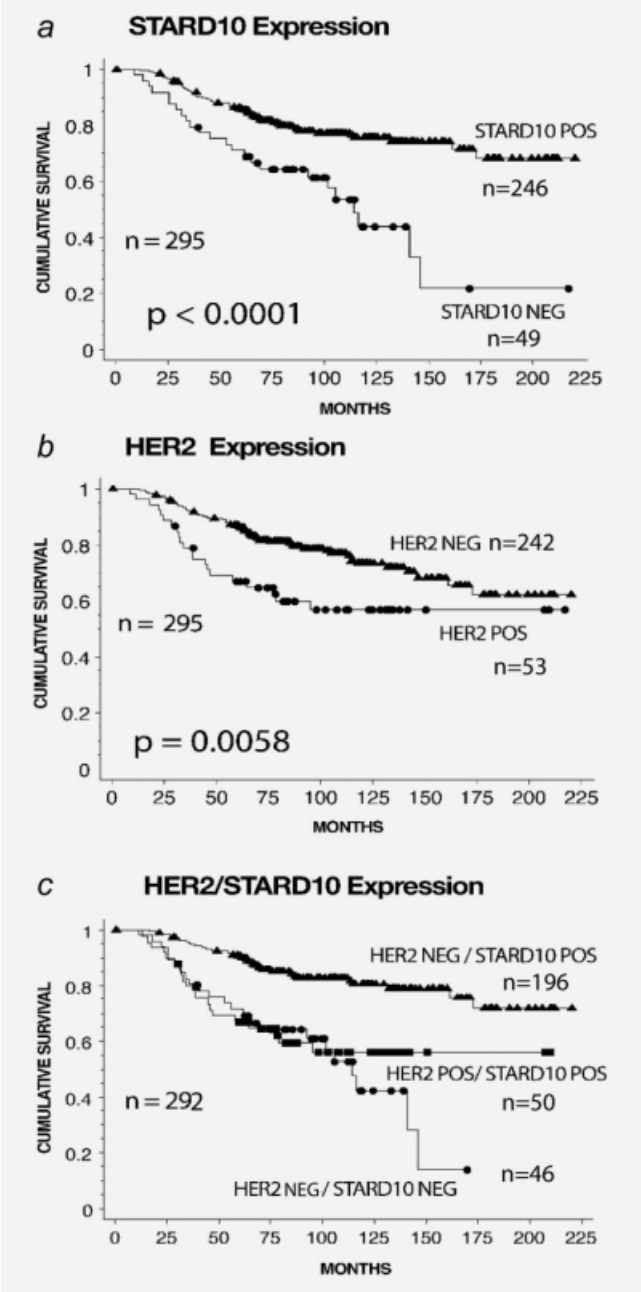

Figure 1. Disease-specific survival according to *STARD10* and *HER2* mRNA levels.

STARD10 was apparent in approximately 15% of breast cancer samples using immunohistochemistry in a preliminary analysis, a cutpoint was identified to dichotomize these data and define a similar proportion. This cutpoint (absolute value –0.210) identified a group of 49 patients (17%) with low expression of *STARD10* mRNA that was associated with a poor disease specific survival (logrank $p < 0.0001$; Fig. 1*a*). Differential STARD10 expression was not associated with tumor size >2 cm (Chi Square $p = 0.53$) or lymph node metastases ($p = 0.22$) but was associated with ER or PR negative tumors ($p < 0.0001$ for each).

As a preliminary study had revealed that 4 of 8 cancers with strong membranous staining of HER2 were also STARD10 positive,[15] we determined the relationship between *STARD10* and *HER2* mRNA. Regression analysis failed to demonstrate any significant relationship between *HER2* and *STARD10* mRNAs as continuous variables ($R = 0.17$). When we selected the upper 35% of *STARD10* mRNA expression, in line with the earlier estimate of STARD10 overexpression by immunohistochemistry,[15] we again failed to demonstrate a significant association with high *HER2* mRNA expression. Most notably, however, only 3 of 295 cancers (1%) had both high *HER2/neu* mRNA and low *STARD10* mRNA expression, while 53 of 295 (18%) tumors were classified as having high *HER2/neu* mRNA; a proportion comparable to the level of *HER2/neu* amplification observed in this (Table 1) and other studies.[20] At this cut-point high *HER2/neu* mRNA was associated with poor patient survival ($p = 0.0058$; Fig. 1*b*). Hence, 46 of a possible 49 cancers with low *STARD10* mRNA expression were *HER2/neu mRNA* negative, and 50 of a possible 53 *HER2/neu mRNA* positive cancers had high *STARD10* mRNA expression. The identification of these 2 almost mutually exclusive subgroups of patients (*i.e. HER2* positive and *STARD10* negative) with particularly adverse outcomes suggested that they were potentially biologically distinct.

To assess if *STARD10* mRNA was an independent prognostic factor, Cox proportional hazard models were constructed with stepwise removal of redundant variables until resolution. The resolved model (Table 2, Model 1) demonstrates that low *STARD10* mRNA expression was an independent prognostic factor in this cohort when modelled with other clinicopathological markers (Model 1) or HER2 alone (Model 2). Kaplan-Meier analysis based on this model, where *HER2/neu* status is stratified by *STARD10* expression demonstrated that *STARD10* positive/*HER2/neu* negative cancers had the best prognosis, *STARD10* negative/HER2/*neu* positive cancers the worst and *STARD10* positive HER2/*neu* positive cancers characterized an intermediate prognostic group (Fig. 1*c*). The influence of low *STARD10* expression in HER2/*neu* positive cancers was not assessable since this occurred in only 3 cases.

Immunohistochemical analysis of STARD10 and association with patient outcome

We next performed immunohistochemistry on TMAs from an independent cohort of 222 carcinomas to assess if STARD10 status was also capable of identifying patient outcome. Tumor size, tumor grade, lymph node status, ER and PR status, HER2/*neu* immunohistochemical status (*i.e.* 25 of 216, 11.6%) positive and *HER2/neu* amplification by FISH (*i.e.* 43 of 212, 20.2%) positive, were all significantly associated with an adverse patient outcome on univariate analysis (Table 1). The higher rate of *HER-2* positivity as assessed by

FISH includes cases which were equivocal (*i.e.* 2+) by IHC as well as IHC 3+. Assignment of STARD10 positivity required an intensity >1 in >10% of cancer cells. Using these criteria, positive STARD10 immunostaining was observed in 198/222 (89%) of cancers. Staining was typically cytoplasmic and nuclear (Fig. 2). Positive STARD10 expression was significantly associated with ER ($p \leq 0.0016$) and PR positivity ($p \leq 0.0001$) but not with histological grade ($p = 0.4189$), tumor size ($p = 0.2527$), lymph node status ($p = 0.1949$), HER2/*neu* IHC status ($p > 0.9999$) or *HER2*/*neu* FISH ($p = 0.6720$). STARD10 was negatively associated with the triple negative phenotype (*i.e.* 24 of 34 TNP cancers were STARD10 positive, $p < 0.0001$).

Table 2. Multivariate analysis for clinicopathological parameters, hormone receptor status and STARD10 and HER2 mRNA expression

Variable (wound cohort)	Hazard ratio (95% CI)	*p* value
Model 1[1]		
Tumor grade 3	2.22 (1.36–3.62)	0.0014
Tumor size >20 mm	1.94 (1.12–3.37)	0.0180
PR positive	0.55 (0.32–0.93)	0.0253
HER2 positive	1.76 (1.02–3.05)	0.0434
Low STARD10	1.83 (1.04–3.22)	0.0361
Model 2[2]		
HER2 positive	2.35 (1.41–3.91)	0.0010
Low STARD10	2.92 (1.79–4.76)	<0.0001

Wound signature cohort (Ref. 12).
[1]The resolved multivariate model shows that low STARD10 expression is an independent poor prognostic factor. [2]The resolved multivariate model shows that low STARD10 expression is independent of HER2 amplification.

Survival analysis

There was a significant association between low/absent STARD10 expression and breast cancer recurrence, metastasis to distant sites and disease-specific survival on Kaplan–Meier analysis (Fig. 3.). Within this group of low expressors ($n = 24$) there was no significant difference in survival between those tumors which had 0 staining ($n = 9$) and those with 1–10% ($n = 15$, $p = 0.507$). As previously reported both HER2/*neu* overexpression by immunohistochemistry and *HER2*/*neu* amplification were associated with an adverse disease-specific survival ($p < 0.0001$ in both cases). When patients were stratified into 4 groups based on STARD10 immunohistochemistry and *HER2* amplification similar results to those derived from the mRNA data were evident. The majority of patients ($n = 153$) were in the STARD10+/HER2− group and had the best prognosis (Fig. 3*d*). *HER2* amplification was associated with poor survival in both the STARD10+ ($n = 38$) and STARD10− ($n = 5$) groups. However, loss of STARD10 expression in the absence of *HER2* amplification ($n = 16$) conferred a poor prognosis similar to that of *HER2* amplification. *HER2* FISH data was only

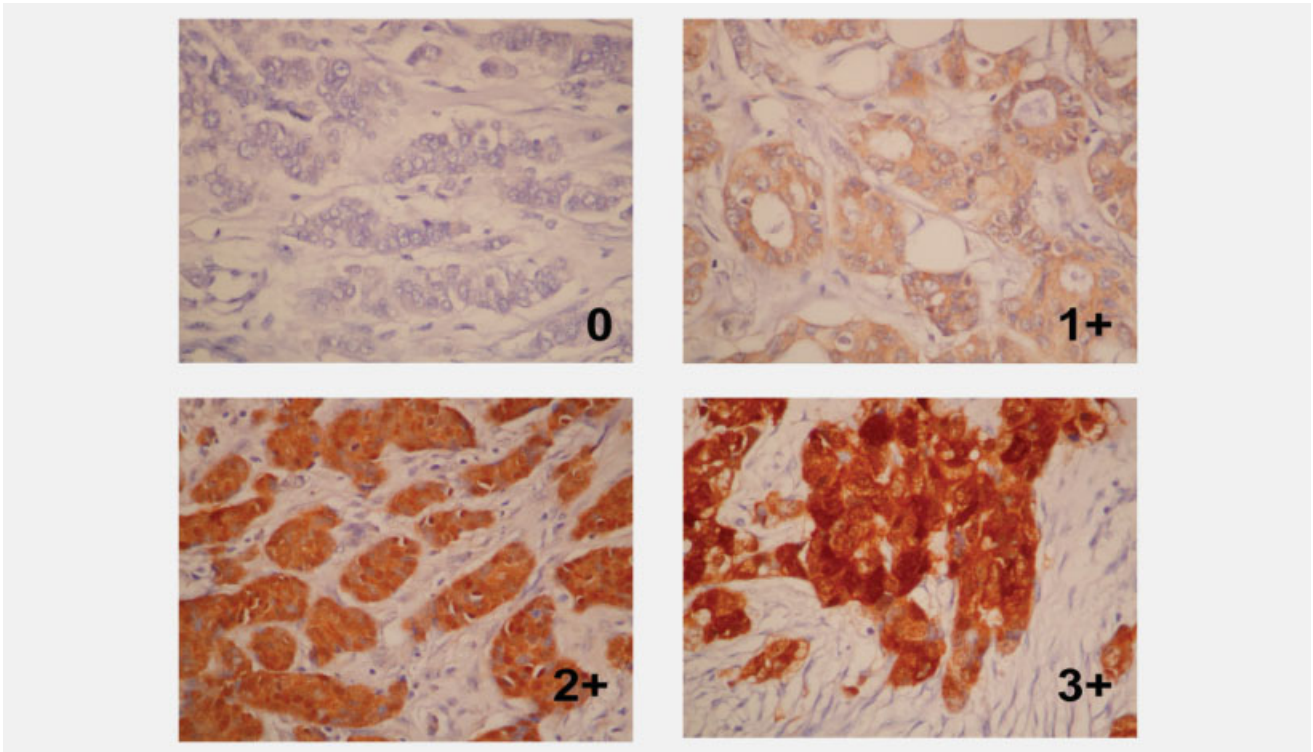

Figure 2. Representative images of STARD10 immunohistochemistry showing no (0), low (1+), medium (2+) and high (3+) intensity staining.

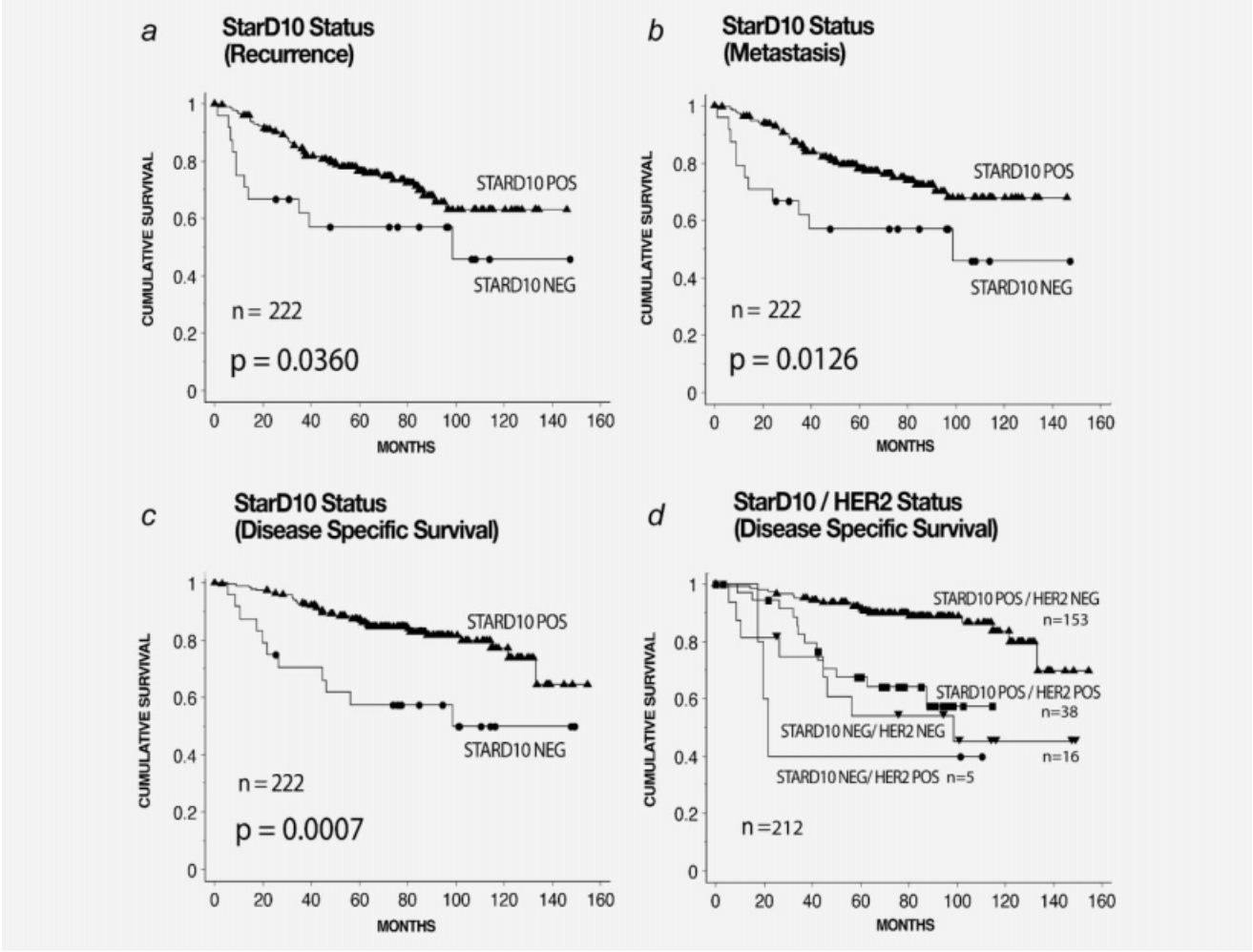

Figure 3. Relationship between STARD10 expression as assessed by immunohistochemistry and (*a*) breast cancer recurrence, (*b*) metastasis and (*c*) disease specific survival alone or in (*d*) combination with *HER2* amplification status.

available on 212 patients; hence, results are not for the full cohort of 222 patients.

Cox univariate analysis confirmed that loss/absence of STARD10 expression was significantly associated with decreased breast cancer-specific survival (HR: 3.065, 95%CI: 1.549–6.066, $p = 0.0013$). Tumor size and grade, lymph node status, ER, PR and HER2 status were also highly significant prognostic markers in this cohort. In a multivariate analysis incorporating these markers (Table 3, Model 1), step-wise elimination of noncontributory/redundant variables was employed until resolution. Using this approach, lymph node involvement, PR status, *HER2* amplification and low STARD10 remained independent predictors of death from breast cancer (Table 3, Model 2). Thus, STARD10 status by immunohistochemistry is an independent predictor of death from breast cancer in this cohort validating the mRNA data from the independent cohort presented in Table 2. In a multivariate model incorporating only STARD10 status and *HER2/neu* amplification, both variables remain independent prognostic factors (Table 3, Model 3). This implies that STARD10 and *HER2/neu* status both provide important clinical prognostic information but identify independent high-risk groups.

To assess the influence on outcome of a triple negative phenotype in this cohort, we replaced STARD10 with triple negative phenotype in the resolved model and confirmed the independent prognostic nature of this phenotype (Table 3, Model 4). However, when STARD10 and triple negative phenotype were assessed together with the other significant variables (Table 3, Model 5), triple negative phenotype was no longer independently prognostic but when modeled together low STARD10 expression and triple negative phenotype remained independent prognostic factors (Table 3, Model 6). Thus, low STARD10 status identifies a poor prognosis group independent of both *HER2* amplification and triple negative phenotype.

Discussion

STARD10 was originally identified in mammary tumors from *Neu/ErbB2* transgenic mice, subsequently isolated from a *c-*

Table 3. Multivariate analysis for breast cancer-specific death for clinicopathological parameters, STARD10 and HER2 status

Variable (Garvan cohort)	Hazard ratio (95% CI)	*p* value
Model 1[1]		
Tumor grade 3	1.964 (0.830–4.647)	0.1243
Tumor size >20 mm	1.789 (0.930–3.445)	0.0816
Lymph node positive	2.129 (1.055–4.295)	0.0349
ER positive	1.039 (0.468–2.307)	0.9251
PR positive	0.304 (0.127–0.731)	0.0078
HER2 amplified	2.173 (1.103–4.280)	0.0249
Low StarD10	2.843 (1.330–6.075)	0.0070
Model 2[2]		
Lymph node positive	2.33 (1.21–4.51)	0.0119
PR positive	0.22 (0.11–0.46)	<0.0001
HER2 amplified	2.67 (1.39–5.11)	0.0031
Low StarD10	2.56 (1.27–5.18)	0.0086
Model 3[3]		
HER2 amplified	3.19 (1.69–6.02)	0.0003
Low StarD10	3.53 (1.77–7.09)	0.0003
Model 4[4]		
Lymph node positive	2.57 (1.31–5.02)	0.0059
PR positive	0.32 (0.14–0.73)	0.0066
HER2 amplified	4.59 (2.05–10.3)	0.0002
Triple negative	2.59 (1.07–6.22)	0.0340
Model 5[5]		
Lymph node positive	2.57 (1.31–5.02)	0.0059
PR positive	0.31 (0.14–0.70)	0.0051
HER2 amplification	3.93 (1.73–8.92)	0.0011
Low StarD10	2.18 (1.06–4.50)	0.0348
Triple negative	2.13 (0.86–5.27)	0.1037
Model 6[6]		
Low StarD10	2.84 (1.38–5.81)	0.0044
Triple negative	2.37 (1.22–4.64)	0.0113

[1]Multivariate analysis of all significant factors associated with outcome identified using univariate analysis shows that low STARD10 is an independent prognostic factor. [2]The resolved multivariate model, following elimination of noncontributory variables (grade 3, tumor size, ER). [3]Low STARD10 is still prognostic and independent of HER2 amplification. [4]Triple negative status is an independent prognostic factor. [5]Triple negative status is not independent when STARD10 is added to the model. [6]A bivariate model shows that STARD10 and triple negative status are independent of each other.

ERBB2 amplified, breast cancer cell line[15] and demonstrated to be preferentially expressed in breast cancer cells overexpressing *c-erbB2*. Furthermore, in 79 invasive breast cancers 28 (35%) demonstrated moderate to intense staining while normal breast tissue was negative. Together with *in vitro* studies, demonstrating that coexpression of STARD10 and the EGFR/*c-erbB1* enhanced anchorage-independent growth,[15] these data led to the hypothesis that *STARD10* and *c-erbB* signaling may cooperate in breast cancer development and progression.

To test this concept we first assessed relationships between *STARD10* and HER2 mRNA levels in a cohort of 295 patients where the oligonucleotide expression array and clinicopathological data were in the public domain.[17] This demonstrated that *STARD10* and HER2/*neu* mRNA were overexpressed in a significant proportion of breast cancers but their expression levels were not correlated. When each set of gene expression data was dichotomized for high and low mRNA expression, low *STARD10* and high HER2/*neu* formed 2 almost mutually exclusive subgroups each of which had a poor disease outcome.

To validate these findings and assess the potential clinical utility of a simple immunohistochemical test for STARD10, we analyzed the expression of STARD10 in an independent cohort of 222 primary breast cancers. Employing a cut-off for positive STARD10 staining as a staining intensity >1 in >10% of cells we identified expression in 198 of 222 (89%) tumors; 24 cases (11%) were classified as low/absent expression. In agreement with the mRNA data, STARD10 expression by immunohistochemistry was significantly associated with ER and PR status but not with tumor size, lymph node status or HER2/*neu* status. Univariate analysis identified absent/low STARD10 expression as a significant predictor of an adverse patient outcome. In a multivariate analysis incorporating STARD10 status with established prognostic factors, STARD10 status remained an independent prognostic factor of disease recurrence, distant metastasis and breast cancer-specific death. When modeled together with *HER2/neu* status, absent/low STARD10 expression remained a significant prognostic factor. This was also true when modeled against the triple negative phenotype which is a well-documented poor prognosis group.[10,11] Thus, this study demonstrates for the first time that immunohistochemical assessment of STARD10 provides clinically important prognostic information, which is independent of other established prognostic factors. Recent gene expression profiling studies have identified molecular subtypes of breast cancer with markedly different prognoses. Established poor prognosis groups include those with HER2 amplification, the HER2 subtype and the basal-like subtype.[2] The triple negative phenotype was a proposed surrogate marker of the basal subtype,[10] but it is now not thought to be synonymous with this group.[11] Interestingly, the low/absent STARD10 group appears to be independent of these molecular subtypes and studies are underway to determine if this group also has a unique molecular signature.

The known function of *c-erbB2* in mammary carcinogenesis[21] and breast cancer and the enrichment of tumors with BRCA1 and p53 mutations and features of stem cells with the basal subtype[10] provide some potential mechanistic basis for these different phenotypes. In contrast, little is known of the normal physiological function of STARD10 in mammary epithelium and breast cancer and whether or not it might be mechanistically involved in the disease process or is merely a

surrogate marker of a more relevant underlying biological process. STARD10 is a member of the START domain proteins, it was initially discovered on the basis of cross reactivity with a phosphoserine-specific antibody in mammary tumors from *neu*/*ErbB2* transgenic mice and was subsequently isolated from SKBR3 human breast carcinoma cells.[15] Recently, STARD10 has been found to function as a dual specificity phospholipid transfer protein for phophatidylcholine (PC) and phosphatidylethanolamine (PE).[22] By binding PC and PE, STARD10 may function in the transfer of these lipids between subcellular compartments and the regulation of cellular signaling events. Deregulation may result in aberrant lipid signaling contributing to cellular transformation. STARD10 is widely expressed and synthesized constitutively in many organs, including the liver, where a putative function is the export of phospholipids into bile. STARD10 expression is also regulated during development in the testes and mammary glands.[23] In the mammary gland, *STARD10* expression is developmentally regulated with highest expression occurring during gestation and lactation.[15] This suggests a role for STARD10 in the enrichment of lipids in milk and as a potential marker of differentiation. In addition, CK2 (casein kinase 2) a gene involved in Wnt/β-catenin signaling[24] and overexpressed in breast cancer[25] downregulates the lipid transfer activity of STARD10 by phosphorylation of serine 284.[26] This may explain a possible biological mechanism responsible for the loss of STARD10 expression in breast cancer and suggests the need for further investigation of its potential role in tumorigeneis that may be independent of its established lipid-binding transport function.[22] Alternatively, *STARD10*'s apparent involvement in breast tumorigenesis may be related to its role as an estrogen target gene.[27] In this regard, a recent study on the molecular response to aromatase inhibitors in a neoadjuvant treatment paradigm identified *STARD10* as one of the most significantly down-regulated genes following treatment.[28] Thus, loss of STARD10 expression may reflect a loss of ER or ER function in agreement with data presented here on the relationship with ER status, and/or a loss of steroid-regulated differentiated cell function *i.e.* dedifferentiation which is a known facet of cancer progression. However, STARD10 immunohistochemical expression was not correlated with tumor grade and remained independently prognostic in a model incorporating PR status and thus does not merely reflect morphological differentiation or hormone-responsiveness. It is interesting to note, however, that the prognostic significance of loss of STARD10 in breast cancer has parallels with loss of AZGP1 in prostate cancer.[29] Both are steroid-regulated genes in the more differentiated, hormone-responsive phases of the disease[30] but when expression is lost, identify patients with a high probability of relapse, metastasis and death. The molecular basis of these interactions remains to be defined.

In conclusion, loss of STARD10 expression in breast cancer strongly predicts an aggressive disease course independent of HER2/*neu* amplification and the triple negative phenotype. These data raise the possibility that loss of expression of STARD10 may provide an additional simple immunohistochemical marker of disease outcome in breast cancer by identifying a subgroup of patients with a particularly adverse outcome.

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