EUROPEAN JOURNAL OF CANCER 44 (2008) 1541–1551

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Forkhead-box A1 (FOXA1) expression in breast cancer and its prognostic significance

Hany Onsy Habashy[a,d], *Desmond G. Powe*[a], *Emad A. Rakha*[a], *Graham Ball*[b], *Claire Paish*[a], *Julia Gee*[c], *Robert I. Nicholson*[c], *Ian O. Ellis*[a,*]

[a]*Department of Histopathology, School of Molecular Medical Sciences, Nottingham University Hospitals NHS Trust, University of Nottingham, Nottingham, UK*
[b]*Division of Life Sciences, Nottingham Trent University, Nottingham, UK*
[c]*Welsh School of Pharmacy, Cardiff University, Cardiff, UK*
[d]*Department of Histopathology, Faculty of Medicine, Mansoura University, Egypt*

ARTICLE INFO

Article history:
Received 19 February 2008
Received in revised form
15 April 2008
Accepted 25 April 2008
Available online 4 June 2008

Keywords:
Breast carcinoma
FOXA1
Oestrogen receptor
Prognosis

ABSTRACT

The forkhead-box A1 (FOXA1) controls downstream transcription of oestrogen receptor (ER)-regulated genes. In this study, the biological and prognostic value of FOXA1 expression was assessed immunohistochemically in a large and well-characterised series of invasive breast carcinoma with a long term follow-up using tissue microarray. FOXA1 expression was associated with steroid hormone receptors (ERα, PgR and AR), other variables of good prognosis such as smaller tumour size, lower histological grade, luminal cytokeratins (CK18 and CK7/8), BRCA1 and E-cadherin. Its expression showed an inverse relation with basal CKs (CK14 and CK5/6) and P-cadherin. We found an association between high FOXA1 expression and a better survival in the whole series however; multivariate analysis showed that FOXA1 was not an independent prognostic marker.

In conclusion, our results show that FOXA1 protein is associated with markers of good prognosis supporting its role as a growth repressor in breast cancer. In this series, FOXA1 was found not to be of an independent prognostic significance in breast cancer and as such its immunohistochemical assessment alone does not appear to have relevance in routine practice to stratify ER-positive (luminal-like) tumours into clinically significant subgroups.

1. Introduction

The forkhead-box A1 (FOXA1) gene is a member of the fox family of transcription factors, expressed in the breast, liver, pancreas, bladder, prostate, colon and lung and can bind to the promoters of more than hundred genes associated with regulation of cell signalling and the cell cycle. [1,2] It is involved in the pathogenesis of many cancers including lung and prostate cancer.[2] In prostate cancer, FOXA1 plays a growth inhibitory role and its expression is associated with markers of differentiation. Previous studies have shown that FOXA1 can act either as a growth stimulator or as a repressor. As a stimulator, it functions as a pioneer factor that binds to chromatinised DNA, opens the chromatin and enhances binding of oestrogen receptor-alpha (ERα) to its target genes.[3] Emphasising its importance, FOXA1 is required for the expression of 50% of ER-regulated genes.[3,4] Using an *in vitro* model, down-regulation of FOXA1 by RNA interference significantly suppressed proliferation of ErbB2-negative and FOXA1-positive breast cancer cell lines.[5]

* *Corresponding author:* Tel.: +44 0 115 9691169; fax: +44 0 115 9627768.
E-mail address: ian.ellis@nottingham.ac.uk (I.O. Ellis).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.04.020

As a repressor, it has been shown that FOXA1 overexpression can block the metastatic progression by influencing expression of the BRCA1 associated cell cycle inhibitor, p27 and promoting E-cadherin expression. This suggests that FOXA1 plays important roles in the upregulation of genes that reduce the growth and motility of breast cancer cells.[6,7]

Importantly, recent global gene expression studies of breast cancer revealed that high FOXA1 mRNA expression is often found in association with ER positivity, and frequently present in a subset of ER-positive tumours that have a favourable outcome (luminal A tumours).[8,9] Therefore, FOXA1 expression appears to have potential relevance in the subclassification of luminal/ER-positive tumours into two subgroups with different biological behaviour and prognosis corresponding to the luminal A and B classes identified in gene expression profiling studies.[8,9]

In this study, FOXA1 protein expression was investigated in the largest series of breast cancers examined to date (696) using high throughput tissue microarrays (TMAs) and immunohistochemistry. Clinicopathological, therapy and outcome information, as well as data on different biomarkers of strong relevance to breast cancer and to FOXA1 protein regulation were available. Data analyses were performed in order to assess the biological and clinical significance of FOXA1 protein expression in unselected primary breast cancer patients as well as in prognostically important subgroups.

2. Materials and methods

2.1. Patient selection

Tissue microarrays (TMAs) were prepared from a series of 880 cases of primary operable breast carcinoma cases from patients age <70 presenting consecutively to the Nottingham Breast Unit, as previously reported[10] This resource has been well characterised and contains patients' clinical and pathological data including patients' age, histological tumour type, primary tumour size, lymph node status, mitotic count and histological grade,[11] Nottingham prognostic index (NPI),[12] vascular invasion (VI), development of recurrence and distant metastases (DM). Survival data including survival time and disease-free interval (DFI) were maintained on a prospective basis. Breast cancer specific survival (BCSS) was defined as the time (in months) from the date of the primary surgical treatment to the time of death from breast cancer. DFI was defined as the interval (in months) from the date of the primary surgical treatment to the first locoregional or distant metastasis. Mean follow-up time was 125 months. The data on other biomarkers with strong relevance to breast cancer and FOXA1 protein including oestrogen receptors (ERα), progesterone receptors (PgR), androgen receptor (AR), BRCA1, p53, EGFR, E-cadherin, P-cadherin, basal and luminal cytokeratins (CKs) [CK5/6, CK14, CK18, CK19 and CK7/8] were available.[10,13–16] HER2 staining was performed by Dr. Jane Starzynski, Heart of England NHS Foundation Trust, Birmingham using the Ventana PATHWAY HER2 (4B5) rabbit monoclonal antibody (Ventana Medical System, AZ, USA). Patient management was based on Nottingham prognostic index (NPI) score and ER status as previously described.[16] Patients' characteristics are summarised in Table 1.

2.2. Immunohistochemistry

Mouse monoclonal antibody to FOXA1 (clone 2F83, ab40868; Abcam, Cambridge, UK) was optimised at a working dilution of 1:2000 using full-face sections of mouse foetal lung tissue as a positive control tissue. Immunohistochemical staining of FOXA1 was carried out using a DakoCytomation Techmate 500 plus (DakoCytomation, Cambridge, UK) automatic immunostainer with a linked streptavidin biotin technique in accordance with the manufacturer's instructions and as previously described [10] after microwave antigen retrieval in citrate buffer (pH 6.0). Negative controls were performed by omitting the primary antibody. Sections were counterstained in haematoxylin and coverslipped using DPX mounting medium.

The H-score (histochemical score) was used to assess the intensity of staining and the percentage of stained cells following immunohistochemistry.[17] Staining intensity was scored from 0, 1, 2 or 3 and the percentage of positive cells at each intensity subjectively estimated to produce a final score in the range 0–300. The cases were scored without

Table 1 – Patients' characteristics

Variable	Number (%)
Age	
<40	40 (5)
40–50	200 (29)
51–60	246 (35)
>60	210 (31)
Tumour size	
<1.5 cm	230 (25)
⩾1.5 cm	460 (75)
LN stage	
1 (negative)	461 (67)
2 (1–3 LN)	158 (22)
3 (>3 LN)	72 (11)
Grade	
1	148 (21)
2	216 (31)
3	325 (48)
NPI	
Poor	96 (14)
Moderate	377 (54)
Good	216 (32)
DM	
No	496 (71)
Positive	194 (29)
Recurrence	
No	403 (58)
Positive	288 (42)
VI	
No	271 (40)
Probable	328 (48)
Definite	82 (12)
ER status	
Negative	213 (33)
Positive	435 (67)

LN = lymph node, NPI = Nottingham prognostic index, DM = distant metastasis and VI = vascular invasion.

knowledge of the patient outcome. The FOXA1 H-score cut-off point for determining positive and negative staining was chosen as the median of the H-score of the informative cases (H-score $\geqslant$ 10). HER2 scoring was performed using the manufacturer recommendations (Ventana Medical System, AZ, USA). Breast carcinomas that were considered positive for HER2 protein overexpression met threshold criteria for the intensity and pattern of membrane staining (2+ or greater on a scale of 0 to 3+) and for the percentage of positive tumour cells (>10%).

2.3. *Statistical analysis*

Statistical analysis was performed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA). Association between the FOXA1 immunoreactivity expression and different clinicopathological parameters was evaluated using Chi-square test. Standard cut-off values for the different biomarkers, needed to determine categorical scores before statistical analysis, were the same as those published in previous studies.[10,13–16] Survival curves were estimated by the Kaplan–Meier method with a log rank test to assess significance. Patients that died due to causes other than breast cancer were considered censored during survival analysis. Multivariate Cox proportional hazard regression model was used to evaluate any independent prognostic effect of the variables with 95% confidence interval. A p-value of <0.05 was considered to reflect a significance.

This study was approved by the Nottingham Research Ethics Committee 2 under the title 'Development of a molecular genetic classification of breast cancer'.

3. Results

After excluding the uninformative TMA cores from the study, 696 tumours were available for assessment. The median age of the patients was 54 years (range 27–70). The staining pattern was nuclear with no evidence of cytoplasmic or membranous staining (Fig. 1A–C). The expression was detected in the nuclei of the malignant cells as well as in some luminal ductal epithelial cells of the entrapped normal tissues in the cores. In the whole series, 55% of the tumours showed a nuclear expression for FOXA1 (H-score $\geqslant$ 10), which varied from weak to strong.

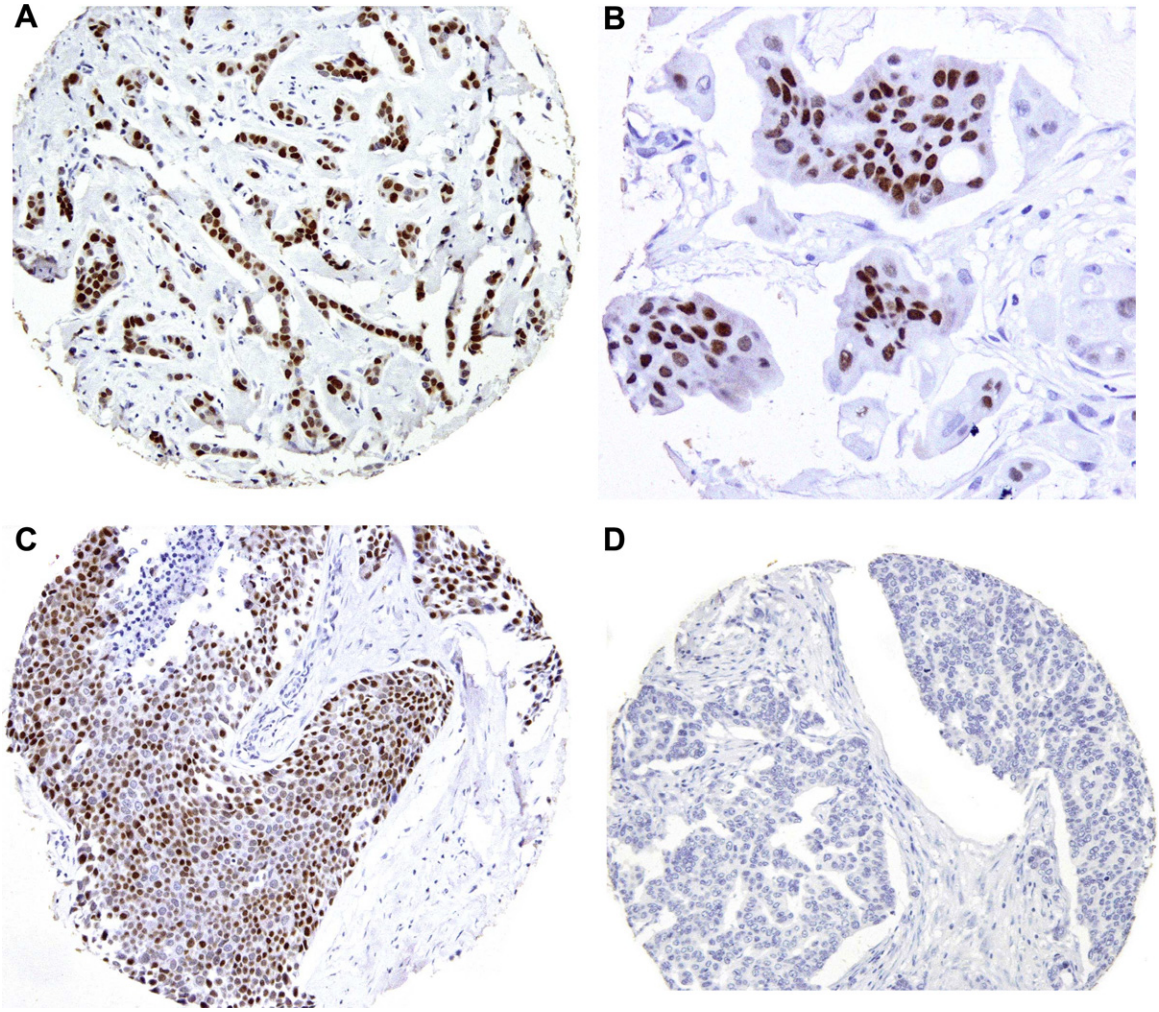

Fig. 1 – Forkhead-box A1 (FOXA1) protein expression in tissue microarray (TMA) cores of (A) lobular, (B) grade II ductal and (C) grade III invasive ductal carcinoma, using immunohistochemistry. (D) A case of grade III invasive ductal carcinoma with negative FOXA1 expression.

3.1. Correlation between FOXA1 expression and clinicopathological variables

FOXA1 expression was associated with smaller primary tumour size ($p < 0.001$), lower grade tumours ($p < 0.001$), lower mitotic count ($p < 0.001$) and good NPI group ($p < 0.001$). No association was found between FOXA1 and patients' age, lymph node stage, vascular invasion, development of recurrence or distant metastasis (Table 2). It also showed an association with histological tumour type with frequent expression in invasive lobular and tubular carcinomas and decreased expression in medullary carcinomas ($p < 0.001$) (Table 3). There was a positive association between FOXA1 expression and ERα ($p < 0.001$), PgR ($p < 0.001$), AR ($p < 0.001$), BRCA1 ($p = 0.003$) and luminal CKs expression. A positive association with E-cadherin expression was found in the whole series ($p = 0.022$) as well as after exclusion of lobular carcinomas, which are typically E-cadherin negative. There was an inverse association between FOXA1 expression and basal CKs expression (CK14; $p = 0.014$ and CK5/6; $p = 0.003$) and P-cadherin ($p = 0.002$). No association was found between FOXA1 and p53, HER2 or EGFR expression (Table 4).

When the analysis was repeated only on the cohort of ER-positive (luminal-like) patients, FOXA1 expression retained its association with smaller tumour size ($p = 0.007$), good NPI ($p = 0.001$) and positive expression of AR ($p = 0.038$) and PgR ($p = 0.016$) but not with any of the other variables (Tables 5 and 6).

When the ER-negative group was separately assessed, no association was found between FOXA1 protein expression and any of the clinicopathological variables included in this study apart from its association with positive AR ($p < 0.001$), CK7/8 ($p = 0.021$) and CK18 expression ($p = 0.012$). Furthermore, when FOXA1 expression was assessed in relation to the different molecular classes of breast cancer as defined by immunohistochemistry (IHC),[10] frequent positive expression was found in luminal tumour classes ($\chi^2 = 11.01$, $p = 0.05$).

Table 2 – Relation of FOXA1 immunostaining with various clinicopathological parameters in the whole breast cancer series

Variable	Total	Negative FOXA1	Positive FOXA1	χ^2	p-Value
Age					
<40	40	19	21	2.822	0.420
40–50	200	97	103		
51–60	246	111	135		
>60	210	185	125		
Tumour size					
<1.5 cm	230	150	80	14.755	<0.001
⩾1.5 cm	460	231	229		
LN stage					
1 (negative)	461	201	260	0.568	0.133
2 (1–3 LN)	158	75	83		
3 (>3 LN)	72	35	37		
Grade					
1	148	50	98	38.209	<0.001
2	216	74	142		
3	325	187	138		
NPI					
Poor	96	53	43	38.515	<0.001
Moderate	377	198	179		
Good	216	60	156		
DM					
No	496	214	282	2.647	0.104
Positive	194	97	97		
Recurrence					
No	403	175	228	0.979	0.322
Positive	288	136	152		
VI					
No	271	125	146	1.501	0.472
Probable	328	141	187		
Definite	82	41	41		
Mitotic counts					
1	218	69	149	30.797	<0.001
2	125	51	74		
3	309	172	137		

LN = lymph node, NPI = Nottingham prognostic index, DM = distant metastasis and VI = vascular invasion.

Table 3 – Distribution of FOXA1 expression according to the histological tumour type

Tumour type	Total	Negative FOXA1	Positive FOXA1	χ^2	*p*-Value
Ductal/NST	382	198	184	33.724	<0.001
Lobular	62	17	45		
Tubular and Tubular mixed	163	56	107		
Medullary	21	16	5		
Other special types[a]	14	6	8		
Mixed[b]	40	15	25		

a Includes Mucoid, invasive cribriform and invasive papillary carcinoma.
b Includes ductal/NST mixed with lobular or special types.

Table 4 – Relation of FOXA1 expression to other biomarkers in whole series

Variable	Total	Negative FOXA1	Positive FOXA1	χ^2	*p*-Value
ERα					
Negative	213	141	72	54.677	<0.001
Positive	435	154	281		
AR					
Negative	245	151	94	42.192	<0.001
Positive	359	125	234		
PgR					
Negative	302	178	124	35.965	<0.001
Positive	340	120	220		
BRCA1					
Negative	73	47	26	8.537	0.003
Positive	425	195	230		
HER2					
Negative	440	205	235	0.008	0.928
Positive	89	41	48		
P53					
Negative	494	111	383	2.715	0.091
Positive	159	26	133		
EGFR					
Negative	418	194	224	0.697	0.404
Positive	106	54	52		
CK5/6					
Negative	523	224	299	8.987	0.003
Positive	150	85	65		
CK14					
Negative	524	231	293	6.080	0.014
Positive	132	74	58		
CK18					
Negative	348	177	171	5.121	0.024
Positive	237	98	139		
CK19					
Negative	267	112	155	3.087	0.079
Positive	399	195	204		
CK7/8					
Negative	352	184	168	11.894	<0.001
Positive	318	123	195		
E-cadherin					
Negative	280	143	137	5.283	0.022
Positive	360	151	209		
P-cadherin					
Negative	185	73	112	9.624	0.002
Positive	312	168	144		

Table 5 – Relation of FOXA1 expression to other clinicopathological parameters in the ER-positive cohort

Variable	Total	Negative FOXA1	Positive FOXA1	χ^2	*p*-Value
Age					
<40	17	4	13	1.782	0.619
40–50	109	36	73		
51–60	158	60	98		
>60	151	54	97		
Tumour size					
<1.5 cm	167	46	121	7.318	0.007
⩾1.5 cm	268	108	160		
LN stage					
1 (negative)	288	93	195	4.161	0.125
2 (1–3 LN)	104	45	59		
3 (>3 LN)	42	16	26		
Grade					
1	125	40	85	5.261	0.072
2	176	56	120		
3	134	58	76		
NPI					
Poor	48	22	26	14.29	0.001
Moderate	205	86	119		
Good	182	46	136		
DM					
No	322	109	213	1.510	0.219
Positive	109	44	65		
Recurrence					
No	266	94	172	0.008	0.930
Positive	165	59	106		
VI					
No	171	58	113	4.386	0.112
Probable	211	72	139		
Definite	44	22	22		
Mitotic counts					
1	187	57	130	4.124	0.127
2	92	32	60		
3	130	76	54		

LN = lymph node, NPI = Nottingham prognostic index, DM = distant metastasis and VI = vascular invasion.

3.2. *Patients' outcome*

In the whole patient series, an association between loss of FOXA1 expression and shorter breast cancer specific survival (BCSS) was found (Log Rank (LR) = 6.987, $p = 0.008$) (Fig. 2). However, multivariate Cox hazard analysis including tumour size, histological grade, lymph node stage and FOXA1 expression showed that FOXA1 expression was not an independent predictor of survival (Hazard ratio (HR) = 0.891, 95% Confidence Interval (CI) = 0.674–1.178, $p = 0.418$) (Table 7). Interestingly, in a model that included only FOXA1 and ERα expression, FOXA1 did not retain independent significance in contrast to ERα which did. No association between FOXA1 expression and disease-free interval (DFI) was found (LR = 1.687, $p = 0.194$) (Fig. 3). In ER-positive group, we found no significant association between FOXA1 expression and BCSS (LR = 1.793, $p = 0.181$) or DFI (LR = 0.001, $p = 0.981$) (Figs. 4 and 5). In the group of patients who had not received hormonal therapy, FOXA1 expression was associated with more favourable BCSS (LR = 5.497, $p = 0.019$) (Fig. 6). No significant differences in BCSS (LR = 0.188, $p = 0.665$) and DFI (LR = 0.872, $p = 0.350$) were noted in the tamoxifen treated patients in relation to FOXA1 expression.

4. Discussion

In recent years, the hunt for reliable prognostic markers and response indicators to various systemic therapies in breast cancer has dramatically increased, supporting the drive to provide more personalised medicine. Several high throughput techniques including gene expression arrays and tissue microarrays have been used.[10,19] Gene expression profiling studies have classified breast cancer into at least five subtypes with distinct biological and clinical significance.[8,9,20] One of the important findings of these global gene profiling studies is that ER-positive tumours cluster together and share a distinct molecular profile which differs to ER-negative tumours. Subsequently, these ER-positive tumours have been termed 'luminal' as they share common molecular markers with luminal epithelial cells of the normal breast. Closer scru-

Table 6 – Relation of FOXA1 expression to other biomarkers in the ER-positive cohort

Variable	Total	Negative FOXA1	Positive FOXA1	χ^2	p-Value
AR					
Negative	104	45	59	4.315	0.038
Positive	297	95	202		
PgR					
Negative	116	52	64	5.804	0.016
Positive	307	99	208		
HER2					
Negative	297	108	189	0.015	0.902
Positive	34	12	22		
P53					
Negative	370	129	241	1.827	0.177
Positive	75	22	53		
EGFR					
Negative	300	115	185	0.045	0.832
Positive	57	21	36		
CK5/6					
Negative	373	134	239	0.214	0.644
Positive	55	18	37		
CK14					
Negative	362	130	232	0.053	0.818
Positive	56	21	35		
CK18					
Negative	192	65	127	0.964	0.326
Positive	194	75	119		
CK19					
Negative	220	74	146	0.809	0.368
Positive	209	130	79		
CK7/8					
Negative	175	60	115	0.124	0.724
Positive	256	92	164		
E-cadherin					
Negative	176	72	104	3.343	0.061
Positive	245	79	166		
P-cadherin					
Negative	156	54	102	3.005	0.083
Positive	180	79	101		

tiny of the luminal tumour group showed that there are at least two distinct molecular subclasses, termed luminal A and B. Luminal A tumours have a favourable prognosis and are characterised by high expression of estrogen-regulated and -associated genes including FOXA1. Luminal B tumours, although also are ER-positive, exhibit a poorer prognosis approaching that of HER2-positive or basal-like tumours and show reduced levels of ER cluster-associated genes.[21,22] These observations suggest that although ER expression is an important membership driver in luminal type breast cancer, other genes also influence the biology and behaviour of these tumours. Furthermore, luminal classification is inherently more complex with approximately 30% of ER-positive tumours failing to respond to hormone therapy. The reasons behind this are unknown but underline the complex interaction between ER and its associated signalling pathways. FOXA1 has received recent attention because it mediates the expression of 50% of ER-regulated genes.[3] In addition, previous studies on the role of FOXA1 in breast cancer have shown controversial results with both growth inhibition and stimulation.[2,5,6]

In this study, we investigated the expression of FOXA1 protein, using immunohistochemistry in a large and well-characterised cohort of breast cancer cases using TMAs, to evaluate its biological and prognostic role in unselected and ER-defined breast cancer subset. In particular, we investigated whether FOXA1 could be used to subclassify luminal tumours. In unselected breast cancer patients, we found that FOXA1 expression was associated with lower histological grade, lower mitotic counts, smaller tumour size and positive expression of hormone receptor positivity (ERα, PgR and AR) and other luminal markers suggesting its association with the low nuclear grade family of breast cancer.[18] In addition, FOXA1 is associated with positive expression of BRCA1 and E-cadherin,

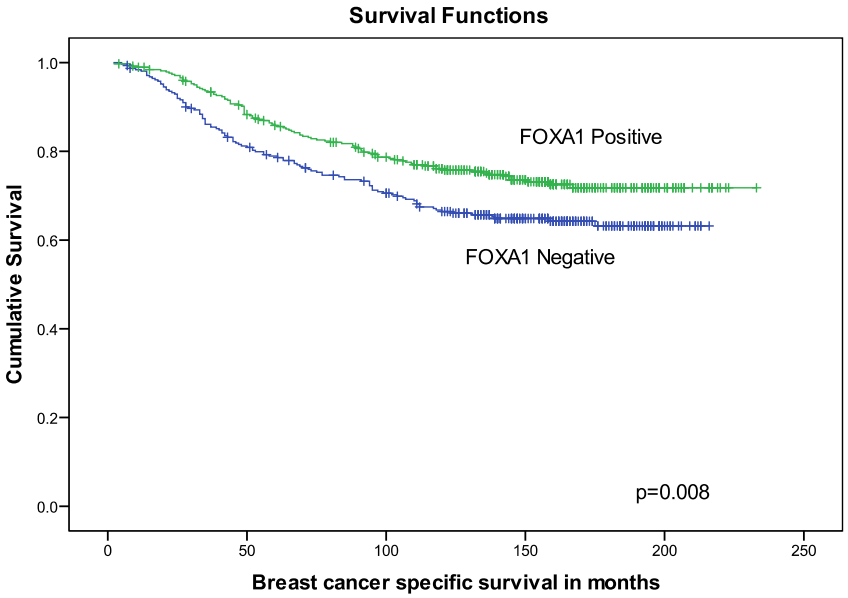

Fig. 2 – Kaplan–Meier plot for FOXA1 protein expression and breast cancer specific survival (BCSS) in whole series (Log Rank, LR = 6.987, $p = 0.008$).

Table 7 – Cox proportional hazards analysis for predictors of breast cancer specific survival: effects of lymph node stage, tumour grade, size, and FOXA1 protein expression in whole series

Variable	Hazard ratio	95% CI	*p*-Value
Lymph node stage	1.802	1.505–2.159	<0.001
Grade	2.024	1.611–2.545	<0.001
Tumour size (⩾1.5 to <1.5 cm)	1.481	1.022–2.146	0.038
FOXA1 expression	0.891	0.674–1.178	0.418

consistent with previous reports proposing that FOXA1 can block the metastatic progression via influencing the BRCA1 associated cell cycle inhibitor p27 expression and regulating E-cadherin expression.[6,7] In support of this hypothesis, although we found that FOXA1 expression did not show a significant association with the development of distant metastasis, there was an inverse relationship trend. Furthermore, FOXA1 expression showed a lack of association with other markers of aggressive tumour phenotype including epidermal growth factor receptors and p53. Moreover, FOXA1 protein expression showed a significant inverse association with the basal phenotype (CK5/6 and CK14 positivity) which is reported to have high proliferative activity and poor prognosis.[13,23] Our findings are in agreement with previous studies showing association of FOXA1 with less-aggressive tumour characteristics.[2,24]

In the ER-positive tumour group, although it was not found to be an independent prognostic marker, FOXA1 showed a trend towards an association with a longer breast cancer spe-

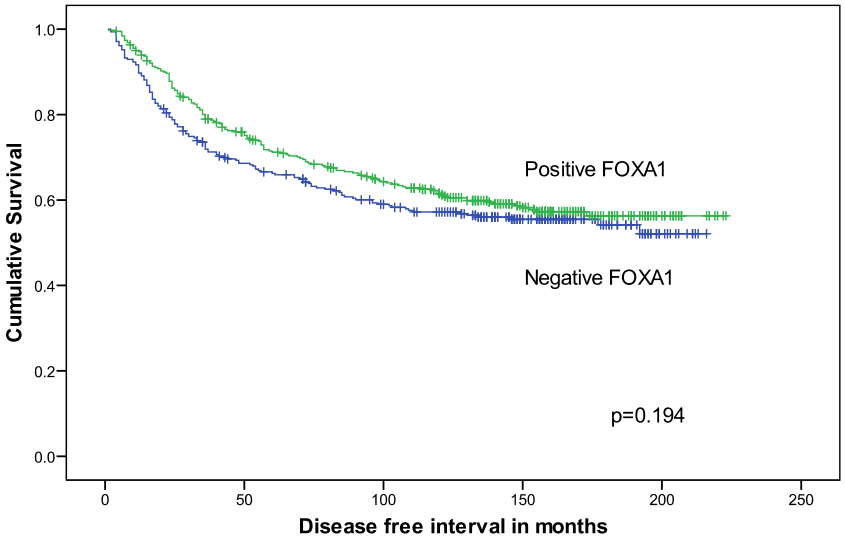

Fig. 3 – Kaplan–Meier plot for FOXA1 protein expression and disease-free interval in whole series (LR = 1.687, $p = 0.194$).

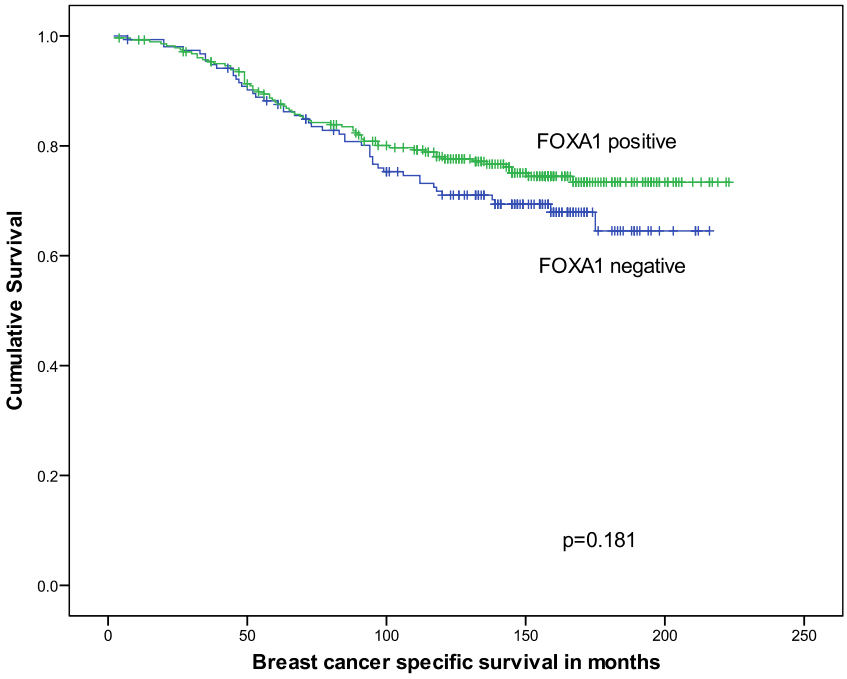

Fig. 4 – Kaplan–Meier plot for FOXA1 protein expression and BCSS in ER-positive cohort (LR = 1.793, $p = 0.181$).

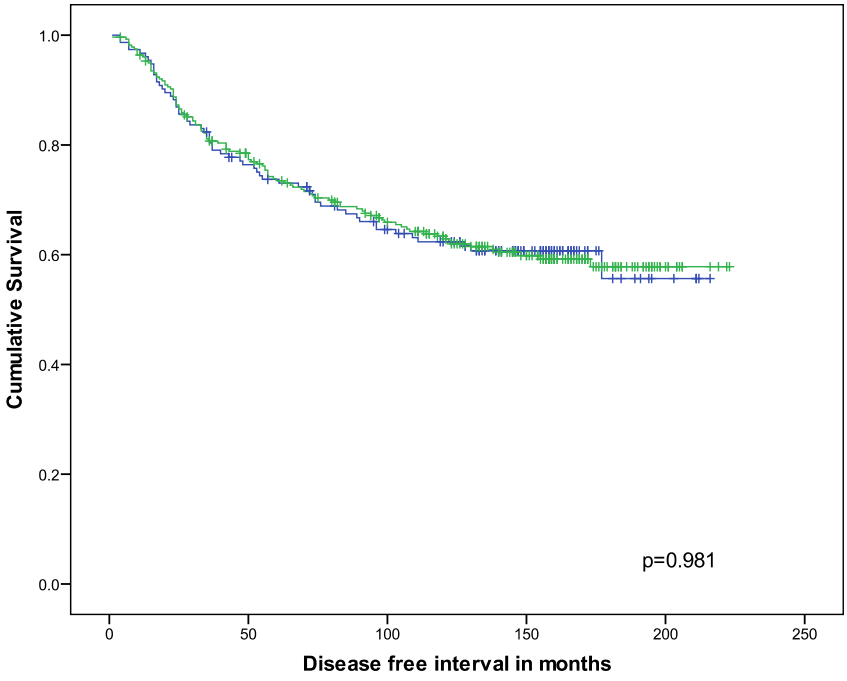

Fig. 5 – Kaplan–Meier plot for FOXA1 protein expression and disease-free interval in ER-positive cohort (LR = 0.001, $p = 0.981$).

cific survival but this was not statistically significant. In ER-negative tumours, FOXA1 was associated with AR expression which is reported to have a good prognostic effect in triple-negative tumours.[25]

In this study, we confirmed the significant positive association between FOXA1 and ERα. Interestingly, we have observed that positive FOXA1 expression is associated with good survival in patients who did not receive hormonal treatment. But, the same association between FOXA1 and good survival outcome was not seen in patients treated with hormonal therapy. This relationship was possibly obscured in treated patients group due to blocking of ERα associated pathways.

An important finding of this study is the lack of prognostic significance of FOXA1 expression as assessed by immunohistochemistry in ER-positive (luminal-like) tumours, thus

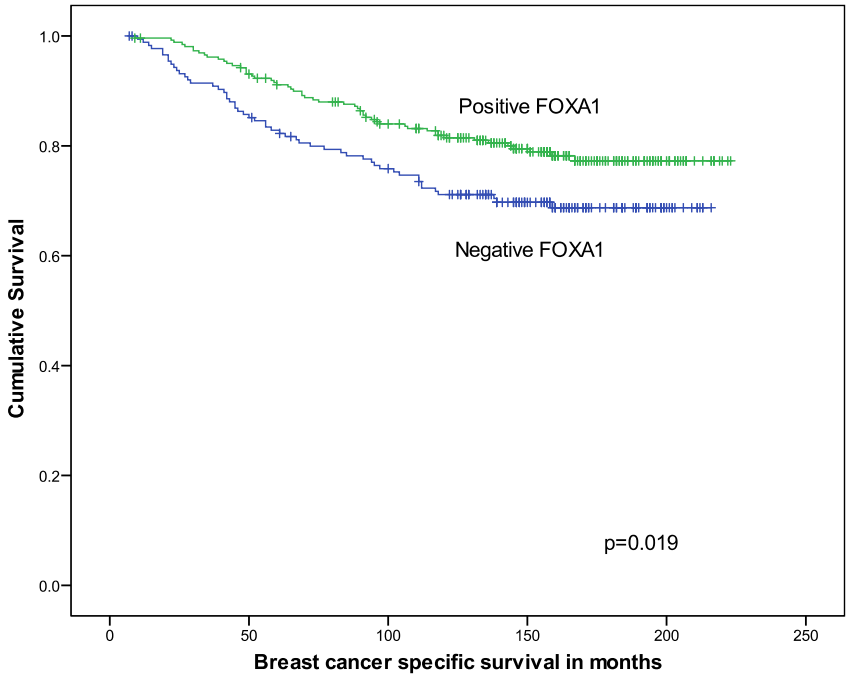

Fig. 6 – Kaplan–Meier plot for FOXA1 protein expression and breast cancer specific survival in patients who have not received hormonal therapy (LR = 5.497, $p = 0.019$).

contrasting with previous results from global gene expression profiling studies, which showed that FOXA1 expression is a feature of luminal A tumours. These results may support the view that the translation of gene expression profiling studies into clinical practice should be interpreted with care and individual markers may not show the same significance when studied in isolation. Another explanation may be the difference in the downstream technique used (RNA expression profiling as opposed to protein expression in immunohistochemistry) and sensitivity of the detection system.

In conclusion, our results support the growth inhibitory role of FOXA1 in breast cancer and emphasised its potentially important biological role. Moreover, we confirmed the strong association between FOXA1 and ERα. In this series, FOXA1 was found not to be of an independent prognostic significance in breast cancer and as such its immunohistochemical assessment alone does not appear to have relevance in routine practice to stratify ER-positive (luminal-like) tumours into clinically significant subgroups. Further work is required to determine if FOXA1 has a specific role in the biological stratification of luminal cancers and in determining therapeutic strategies.

Conflict of interest statement

None declared.

Acknowledgements

The authors thank Dr. Jane Starzynski, Heart of England NHS Foundation Trust, Birmingham for the HER2 immunostaining. Hany Habashy is funded by the Egyptian Cultural Bureau in London. Graham Ball is funded by the John and Lucille van Geest Foundation.

REFERENCES

1. Lin L, Miller CT, Contreras JI, et al. The hepatocyte nuclear factor 3 alpha gene, HNF3 alpha (FOXA1), on chromosome band 14q13 is amplified and overexpressed in esophageal and lung adenocarcinomas. *Cancer Res* 2002;**62**:5273–9.
2. Wolf I, Bose S, Williamson EA, Miller CW, Karlan BY, Koeffler HP. FOXA1: growth inhibitor and a favorable prognostic factor in human breast cancer. *Int J Cancer* 2007;**120**:1013–22.
3. Laganiere J, Deblois G, Lefebvre C, Bataille AR, Robert F, Giguere V. Location analysis of estrogen receptor alpha target promoters reveals that FOXA1 defines a domain of the estrogen response. *Proc Nat Acad Sci USA* 2005;**102**:11651–6.
4. Carroll JS, Brown M. Estrogen receptor target gene: an evolving concept. *Mol Endocrinol* 2006;**20**:1707–14.
5. Yamaguchi N, Ito E, Azuma S, et al. FoxA1 as a lineage-specific oncogene in luminal type breast cancer. *Biochem Biophys Res Commun* 2008;**365**:711–7.
6. Williamson EA, Wolf I, O'Kelly J, Bose S, Tanosaki S, Koeffler HP. BRCA1 and FOXA1 proteins coregulate the expression of the cell cycle-dependent kinase inhibitor p27(Kip1). *Oncogene* 2006;**25**:1391–9.
7. Liu YN, Lee WW, Wang CY, Chao TH, Chen Y, Chen JH. Regulatory mechanisms controlling human E-cadherin gene expression. *Oncogene* 2005;**24**:8277–90.
8. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *PNAS* 2001;**98**:10869–74.
9. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in

independent gene expression data sets. *Proc Nat Acad Sci USA* 2003;**100**:8418–23.
10. El-Rehim DMA, Ball G, Pinder SE, et al. High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. *Int J Cancer* 2005;**116**:340–50.
11. Elston CW, Ellis IO. Pathological prognostic factors in breast-cancer 1. The value of histological grade in breast-cancer – experience from a large study with long-term follow-up. *Histopathology* 1991;**19**:403–10.
12. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham prognostic index in primary breast-cancer. *Breast Cancer Res Treat* 1992;**22**:207–19.
13. Rakha EA, El-Rehim DA, Paish C, et al. Basal phenotype identifies a poor prognostic subgroup of breast cancer of clinical importance. *Euro J Cancer* 2006;**42**:3149–56.
14. El-Rehim DMA, Pinder SE, Paish CE, et al. Expression of luminal and basal cytokeratins in human breast carcinoma. *J Pathol* 2004;**203**:661–71.
15. Rakha EA, El Rehim DA, Pinder SE, Lewis SA, Ellis IO. E-cadherin expression in invasive non-lobular carcinoma of the breast and its prognostic significance. *Histopathology* 2005;**46**:685–93.
16. Rakha EA, El-Sayed ME, Powe DG, et al. Invasive lobular carcinoma of the breast: response to hormonal therapy and outcomes. *Euro J Cancer* 2008;**44**:73–83.
17. McCarty KS, Miller LS, Cox EB, Konrath J, McCarty KS. Estrogen-receptor analyses – correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med* 1985;**109**: 716–21.
18. Abdel-Fatah T, Powe D, Hodi Z, Reis J, Lee A, Ellis I. Morphological and molecular evolutionary pathways of low and high grade breast cancers and their putative precursor lesions. *Virchows Archiv* 2007;**451**:143.
19. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;**415**:530–6.
20. Sorlie T. Molecular portraits of breast cancer: tumour subtypes as distinct disease entities. *Euro J Cancer* 2004;**40**:2667–75.
21. Sotiriou C, Neo SY, McShane LM, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Nat Acad Sci USA* 2003;**100**:10393–8.
22. Sotiriou C, Wirapati P, Loi S, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 2006;**98**:262–72.
23. Rakha EA, El-Sayed ME, Green AR, Paish EC, Lee AHS, Ellis IO. Breast carcinoma with basal differentiation: a proposal for pathology definition based on basal cytokeratin expression. *Histopathology* 2007;**50**:434–8.
24. Badve S, Turbin D, Thorat MA, et al. FOXA1 expression in breast cancer correlation with luminal subtype A and survival. *Clin Cancer Res* 2007;**13**:4415–21.
25. Rakha EA, El-Sayed ME, Green AR, Lee AHS, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. *Cancer* 2007;**109**:25–32.