Nucleostemin expression in breast cancer is a marker of more aggressive phenotype and unfavorable patients' outcome
A STROBE-compliant article

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Abstract

Cancer stem cells (CSCs) are postulated to play significant role in the pathogenesis, progression as well as drug resistance of breast cancer. Nucleostemin (NS) is thought to be a key molecule for stemness, and the clinical impact of NS immunoreactivity in breast cancer can indicate its actual role and future therapeutic potentials.

The current study is an observational study with an attempt to evaluate the correlation between NS expression (protein and gene expression levels) and different clinicopathological attributes of invasive breast cancer. For that reason, we investigated NS immunohistochemistry expression on commercial tissue microarray (TMA) of 102 patients and 51 archival specimens from patients admitted to Saqr Hospital, Ras Al Khaimah and diagnosed in Al Baraha Hospital, Dubai, UAE. In addition, the association between NS (GNL3) gene expression and different prognostic parameters as well as patient outcome was also evaluated using 2 large publicly available databases.

Interestingly, we found NS expression to be associated with less differentiated and more advance stage. In addition, NS expression was significantly higher in larger size ($P=.001$) and LN-positive tumors ($P=.007$). Notably, NS expression was significantly correlated to P53 positive ($P=.037$) status. Furthermore, NS was found to be more expressed in the highly aggressive breast cancer subtypes including human epidermal growth factor receptor 2 (HER-2) and triple negative breast cancer (TNBC) subtypes. Moreover, our results also showed that high GNL3 gene expression to be associated with poor patient outcome and higher chances of tumor recurrence.

Our results highlight NS expression as a marker of aggressive phenotype and poor outcome and indicate its possible use as a potential target for CSC-associated breast cancer management.

Abbreviations: CSC = cancer stem cell, ER = estrogen receptor, GOBO = gene expression based outcome, HER-2 = human epidermal growth factor receptor 2, LN = lymph node, NS = nucleostemin, PR = progesterone receptor, TMA = tissue microarray, TNBC = triple negative breast cancer.

Keywords: breast cancer, G Protein Nucleolar 3, patient outcome, prognosis, stem cells markers

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1. Introduction

Despite all the advances in the oncology field, breast cancer remains one of the leading causes of death in women.[1,2] Moreover, disease recurrence and drug resistance are still major challenges for breast cancer survivors.[3] Hence, identification of new markers that can predict patients with high risk of development of tumor recurrence and drug resistance might help not only in designing more aggressive therapeutic protocols to treat such patients but also in the discovery of novel and more precise therapeutic options.

One of the proposed mechanisms to explain the tumor recurrence is the presence of cancer stem cells (CSCs).[2] Despite the fact that they represent a small proportion of the tumor cells, CSCs are believed to be responsible for tumor initiation and to play a major role in tumor recurrence, metastasis, and drug resistance.[4–6] This might be due to their unique features like self-renewal and pluripotency.[7,8] Thus, investigating markers associated with breast CSCs might provide an important tool to identify patients with higher risk of more aggressive disease behavior.

Nucleostemin (NS) is GTP-binding protein that was shown to be involved in different processes including ribosome synthesis, pre-rRNA processing as well as genome protection.[9–11] The main site of NS accumulation is the nucleoli. Following the

binding of NS with GTP it moves to the nucleoplasm, where it interacts with group of proteins like p53 to execute different functions including cell cycle progression, apoptosis, proliferation as well as self-renewal.[12,13] Indeed, NS was found to be upregulated in breast cancer cell lines and was later proved to play an essential role in stem cells maintenance through regulation of their self-renewal capacity.[14] In addition, it was previously reported that NS gene is markedly expressed in the nucleoli of undifferentiated cells including stem cells compared to more differentiated cells.[15] Moreover, numerous studies confirmed its expression in a wide spectrum of cancers including colon cancer,[16] lung cancer,[17] brain tumors,[18] breast cancer,[12] and hepatocellular carcinoma.[19]

We report here for the first time, the prognostic value of NS expression in a multiethnic cohort of breast cancer patients from United Arab Emirates (UAE) compared to a cohort of commercial tissue microarray (TMA) of breast cancer patients.

Our results revealed an important association between NS protein, as well as, gene expression levels with well-known, poor clinicopathological parameters including larger tumor size, advanced tumor stage, high grade as well as more aggressive breast cancer molecular subtypes. Finally, our results also showed that higher NS gene (GNL3) expression was associated with higher possibility of tumor recurrence. Together, these findings highlight the promising use of NS as a marker of tumor progression and poor patient outcome.

2. Materials and methods

2.1. Patients' cohorts

A total of 431 breast lesions (excluding mastitis and breast abscess) were admitted to the Surgery Department, Saqr Hospital, Ras Al Khiamah, UAE in the period from 1998 to 2011. Ninety-five cases were diagnosed as primary invasive breast carcinoma in the Pathology Department, Al Baraha Hospital, Dubai, UAE, out of which, only 51 cases with available complete records and paraffin blocks with sufficient tissue were included in the study.

The other cohort was a commercial TMA purchased from Pantomics (BRC1021, Richmond, USA) and consisted of 102 cores. It included 5 normal/benign cores, 6 in situ carcinomas and 91 cores of invasive breast carcinoma from different histological and molecular subtypes.

The majority of the patients of the UAE cohort were Asians 21/51 (41.2%), while 12 (23.5%) patients were UAE nationals and 16 (31.4%) were from other Arab countries. For the UAE cohort, the patients involved in the study have given their permission to be involved in this study. The study protocol was approved by the Research and Ethics Committee of RAK Medical and Health Sciences University—UAE. Moreover, for the commercial TMA, the providing company assured that the patients involved in this cohort gave a written informed consent and tissues were collected with high ethical standards

2.2. Immunohistochemistry

The tissue sections were deparaffinized, rehydrated using a series of methanol concentrations, then immersed in hydrogen peroxide solution for 30 minutes to ensure endogenous peroxidase activity blockage. This was followed by heat-induced antigen retrieval with citrate buffer at pH 6.0 for 30 minutes. The slides then incubated with rabbit anti-NS polyclonal antibody (bs-6846R) at 4°C overnight, followed by HRP conjugation. The reaction was visualized with diaminobenzidine and the slides were then counterstained with Mayer's hematoxylin and examined manually.

2.3. Immunohistochemistry scoring

All the cases were evaluated for immunostaining in a blind manner from there clinic-pathological parameters by 3 independent pathologists. All the information related to patient's identification remains anonymous in adherence to the ethical and legal guidelines.

For NS, cases were considered positive if 10% or more tumor cells exhibited nucleolus/nucleoplasm staining. As regards ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), and p53, the assessment of the UAE cohort had been done as previously described.[20] Moreover, the cases were classified into molecular subtypes according to immunohistochemistry surrogate of molecular breast cancer subtypes that was previously used.[21]

2.4. Data mining

Two large, publicly available databases were used in our report; the first is the gene expression-based outcome (GOBO) database, which includes data of 1881 breast cancer patients. This tool enabled us to investigate the association between NS (GNL3) gene expression and various prognostic parameters including grade and molecular subtypes, in addition to its correlation with patient outcome represented as relapse-free survival (RFS). The median (middle quantile) was used for dividing the cases into high or low expression groups as previously described.[22]

We also used Curtis dataset (around 2000 patient) of the ONCOMINE database to investigate the association between NS (GNL3) gene expression and tumor progression presented as tumor stage and also its association with p53 status.

3. Statistical analysis

The association between NS expression and different clinic-pathological parameters were estimated using chi-square test (χ^2) test. $P < .05$ was considered to be statistically significant.

4. Results

4.1. NS expression in 2 different patients' cohorts

Initially, we investigated the expression levels of NS in 2 different patients' cohorts, the UAE cohort (51 cases) and the commercial TMA cohort (102 cases). Our results revealed that NS is expressed in 43.13% of the UAE cohort compared to the 78.35% in the commercial TMA cohort. This difference might be attributed to the fact that patients from UAE cohort had mostly early stage disease compared to more advance disease in the TMA cohort (Tables 1 and 2).

Interestingly, examining the NS status of the entire cohort according to the different histopathological subtypes, revealed that NS was predominantly expressed in ductal carcinomas (70.4%), while lobular carcinomas showed significantly lower expression of NS with only (25%) of cases exhibiting positive NS expression ($P = .027$) (Table 3).

4.2. NS expression is associated with poorly differentiated breast cancer

Keeping in mind the chief role of NS in maintaining stemness and the strong association between stemness and loss of differentiation, we examined the association between NS protein expression and tumor grade. Indeed, in UAE cohort, poorly differentiated (grade III) tumors showed around double (52%) the expression of NS compared to the well differentiated (grade I) tumors (28.57%) (Table 1)(Fig. 1A).

The same trend was also observed in the larger commercial TMA, with the highest NS expression detected in the moderately differentiated and poorly differentiated (grade III) (81.81% and 81.25% respectively) tumors compared to the well differentiated (grade I) tumors (66.67%) (Table 2).

To further analyze this point, we next evaluated the association between NS gene (GNL3) and tumor grade in more than 1881 breast cancer samples using GOBO database. Indeed, GNL3 gene expression showed a significant association ($P = .03$) with poorly differentiated (grade III) tumors compared to well-differentiated tumors (Fig. 1B).

4.3. NS expression is associated with more advanced stage and larger tumor size

Next, we studied the association between NS and other well established clinicopathological parameters including tumor size, lymph node (LN) involvement as well as tumor stage.

<div style="background:#7a7f83;color:white;padding:2px 6px;display:inline-block">Table 1</div>

Association between Immunohistochemical expression of NS and the clinicopathological parameters in the UAE cohort.

	Positive	Negative	Total	% of Positive	P value
Tumor grade					
Grade I	2	5	7	28.57%	.42
Grade II	7	12	19	36.84%	
Grade III	13	12	25	52%	
Tumor stage					
Stage I	8	16	24	33.33%	.46
Stage II	8	8	16	50%	
Stage III	2	3	5	40%	
LN status					
LN Negative	16	23	39	41.02%	.72
LN Positive	2	4	6	33.33%	
Tumor size					
T1	8	17	25	32%	.22
T2 & 3	10	10	20	50%	
ER status					
ER Positive	12	16	28	42.85%	.50
ER Negative	12	11	23	52.17%	
PR status					
PR Positive	5	9	14	35.71%	.60
PR Negative	18	18	36	50%	
HER-2 status					
HER-2 Positive	8	11	19	42.10%	.58
HER-2 Negative	16	16	32	50%	
Molecular subtypes					
Luminal A	6	11	17	35.29%	.64
Luminal B	5	8	13	38.46%	
HER-2	2	3	5	40%	
TNBC	9	7	16	56.25%	
P53 status					
Negative	14	23	37	37.83%	.21
Positive	8	6	14	57.14%	

ER = estrogen receptor, HER-2 = human epidermal growth factor receptor 2, LN = lymph node, NS = nucleostemin, PR = progesterone receptor, TNBC = triple negative breast cancer.

Our results observed more frequent NS positivity with increasing tumor size in both cohorts (Tables 1 and 2). Noticeably, the UAE cohort displayed more NS expression in the LN negative tumors compared to the LN positive tumors (41% and 33.33% respectively) (Table 1), whereas, the larger commercial TMA showed higher NS immunoreactivity in the LN positive tumors (86.67%) compared to the LN negative tumors (71.15%) ($P = .06$) (Table 2).

Overall, assessing the relation between NS expression and tumor stage, which is regarded as a major indicator of disease progression, revealed that, stage II & III tumors of the UAE cohort showed more frequent NS expression (50% and 40% respectively) compared to stage I tumors (33.33%) (Table 1). The same trend was also observed in the commercial TMA with the highest NS expression detected in the more advanced stage III & IV tumors (83.33%) and least expressed in the early stage 0 cases (66.67%) (Table 2).

Interestingly, when we combined cases from both cohorts (Table 3), we found a significant association between NS immunoreactivity and larger tumor size ($P = .001$) and positive LN status ($P = .007$), although this association was not significant when examined in each cohort separately. In addition, NS was also found to be more expressed in the more advanced stages III and IV (78.04%) compared to early stages I and II (61.05%) and in situ (stage 0) (66.67%) tumors of the entire cohort, however, this trend did not reach statistical significance (Table 3) (Fig. 2A).

To further explore this point, we next evaluated GNL3 gene expression in more than 2000 breast cancer samples obtained from Curtis dataset of ONCOMINE database (Fig. 2B). As expected, GNL3 expression was highest in the more advanced stage IV tumors in comparison to the less advanced cases (median = 0.3335) and least in the early stage I disease (median = 0.108).

These findings highlight that NS expression is associated with breast cancer progression and advanced disease.

4.4. NS expression is associated with more aggressive breast cancer subtypes

Considering that breast cancer represents a heterogeneous group of diseases with distinct histological, molecular as well as clinical subgroups, we attempted to investigate the expression levels of NS in association with classical markers like ER, PR, and HER-2 as well as its expression in different breast cancer molecular subtypes.

Interestingly, while NS showed no significant association with the PR and HER-2 status, it showed an important association with estrogen receptor (ER) negative status reaching to significant levels in the commercial TMA ($P = .012$). This association with ER-negative tumor was further supported though our finding that NS expression showed the highest expression in the ER-negative and highly aggressive triple negative breast cancer (TNBC) tumors (56.25%) in the UAE cohort, with lowest expression in the least aggressive, ER-positive, luminal A tumors (35.29%) (Table 1). Furthermore, comparable results were obtained using the larger commercial TMA with the highest NS expression detected in the HER-2 (85.71%) followed by TNBC tumors (81.81%) (Both ER-negative tumors) and the least expression in luminal A tumors (63.15%) (Table 2).

Combined analyses of both cohorts revealed an obvious trend of association between NS expression and the 2 more aggressive (ER-negative) breast cancer subtypes (HER-2 and TNBC) ($P = .09$) (Table 3).
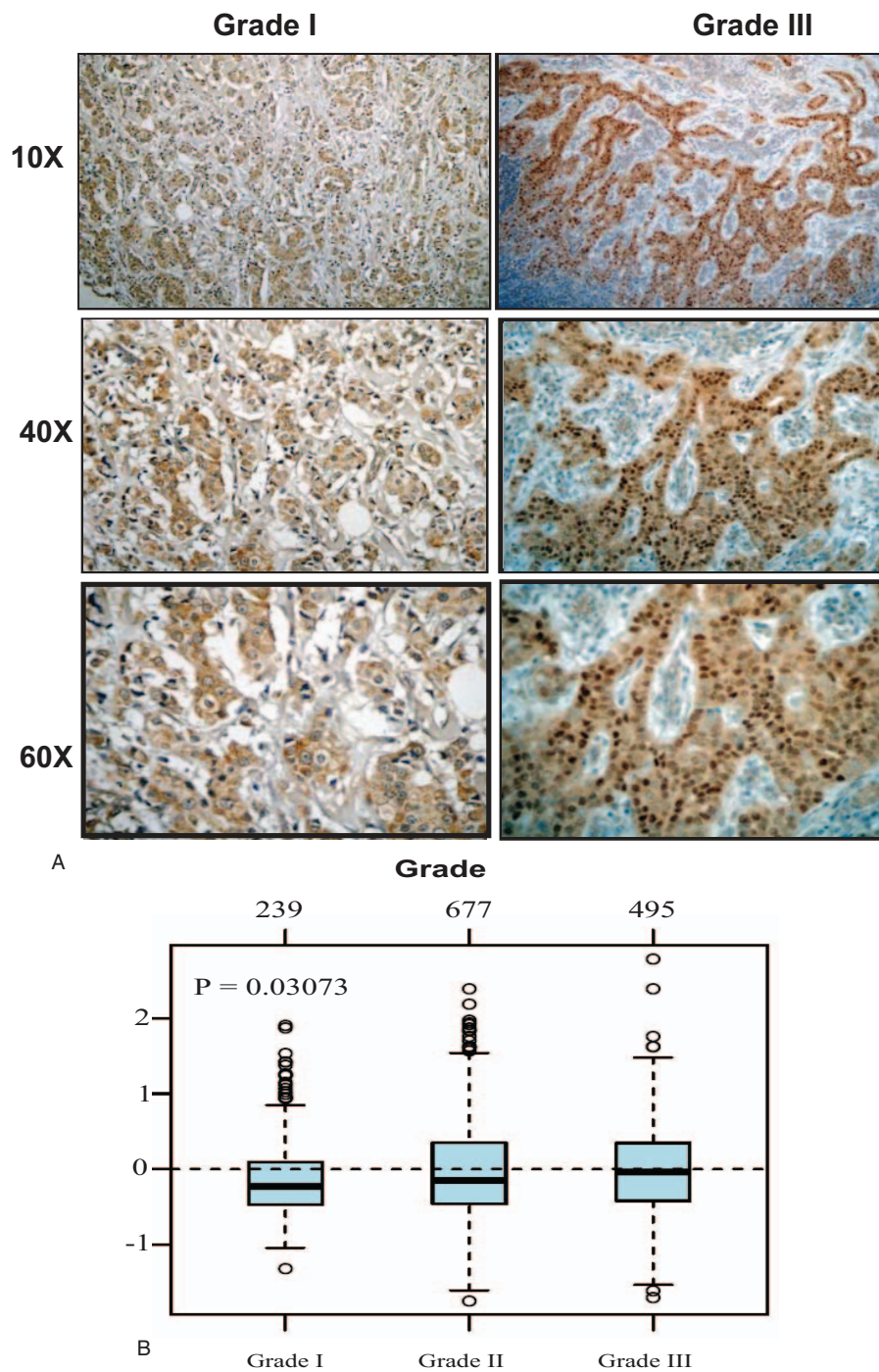
Figure 1. The association between NS expression and tumor grade. (A) Representative pictures of immunohistochemical expression of NS in breast cancer samples of different grades. (B) GNL3 gene expression and its association with tumor grade in 1881 breast cancer samples obtained from GOBO database. GOBO = gene expression based outcome, NS = nucleostemin.

To further clarify the role of NS in different breast cancer subtypes, we next evaluated the expression levels of GNL3 gene expression in different molecular subtypes (PAM 50 classification) using GOBO database. Indeed, GNL3 expression was shown to be significantly higher in TNBC tumors as well as luminal B tumors with the least expression in the luminal A tumors ($P=.02$) (Fig. 2 C). This highlights the strong association between NS and its gene with more aggressive subtypes.

4.5. NS expression is associated with P53 positive expression

Previous studies suggested that NS plays an important role in the tumor suppressor p53 inactivation through its interaction with MDM2 leading to cell cycle progression and cell survival.[23,24] Indeed, p53 is known for its short half-life, however, when it is mutated, usually this leads to its stability and accumulation in cells.

As anticipated, UAE and TMA cohorts demonstrated higher NS expression among p53 positive tumors (57.14% and 87.5%

Table 2

Association between Immunohistochemical expression of NS and the clinicopathological parameters in the commercial TMA.

	Positive	Negative	Total	% of Positive	P value
Histopathology					
Normal & benign	4	1	5	80%	.93
Malignant	76	21	97	78.35%	
Invasiveness					
In situ	4	2	6	66.67%	.47
Invasive	72	19	91	79.12%	
Tumor grade					
Grade I	12	6	18	66.67%	.38
Grade II	45	10	55	81.81%	
Grade III	13	3	16	81.25%	
Tumor stage					
Stage 0	4	2	6	66.67%	.56
Stage I-II	42	13	55	76.36%	
Stage III-IV	30	6	36	83.33%	
LN status					
LN Negative	37	15	52	71.15%	.06
LN Positive	39	6	45	86.67%	
Tumor size					
Tis	4	2	6	66.67%	.33
T1	0	0	0	0	
T2	33	11	44	75%	
T3	30	4	34	88.23%	
T4	9	4	13	69.23%	
ER status					
ER Positive	24	12	36	66.67%	.012
ER Negative	46	6	52	88.46%	
PR status					
PR Positive	25	10	35	71.42%	.16
PR Negative	42	8	50	84%	
HER-2 status					
HER-2 Positive	40	9	49	81.63%	.7
HER-2 Negative	29	8	37	78.37%	
Molecular subtype					
Luminal A	12	7	19	63.15%	.31
Luminal B	16	6	22	72.72%	
HER-2	24	4	28	85.71%	
TNBC	9	2	11	81.81%	
P53 status					
Negative	48	17	65	73.84%	.12
Positive	28	4	32	87.5%	

ER = estrogen receptor, HER-2 = human epidermal growth factor receptor 2, LN = lymph node, NS = nucleostemin, PR = progesterone receptor, TMA = tissue microarray, TNBC = triple negative breast cancer.

Table 3

Immunohistochemical expression of NS in the studied clinico-pathological parameters in both cohorts.

|	Positive	Negative	Total	% of Positive	P value
Histological type					
Ductal	88	37	125	70.4%	.027
Lobular	3	9	12	25%	
Others	4	4	8	50%	
Tumor grade					
Grade I	14	11	25	56%	.38
Grade II	52	22	74	70.27%	
Grade III	25	15	41	62.5%	
Tumor stage					
Stage 0	4	2	6	66.67%	.15
Stage I-II	58	37	95	61.05%	
Stage III-IV	32	9	41	78.04%	
LN status					
LN Negative	53	38	91	58.24%	.007
LN Positive	41	10	51	80.39%	
Tumor size					
Tis	4	2	6	66.67%	.001
T1	8	17	25	32%	
T2	41	17	58	70.68%	
T3	32	8	40	80%	
T4	9	4	13	69.23%	
ER status					
ER Positive	36	28	64	56.25	.11
ER Negative	58	27	85	68.23529	
PR status					
PR Positive	30	19	49	61.22449	.31
PR Negative	60	26	86	69.76744	
HER-2 status					
HER-2 Positive	48	20	68	70.58824	.5
HER-2 Negative	45	24	69	65.21739	
Molecular subtype	36	28	64		
Luminal A	18	18	36	50%	.09
Luminal B	21	14	35	60%	
HER-2	26	7	33	78.78%	
TNBC	18	9	27	66.67%	
P53 status					
Negative	62	40	102	60.78%	.037
Positive	36	10	46	78.26%	

ER = estrogen receptor, HER-2 = human epidermal growth factor receptor 2, LN = lymph node, NS = nucleostemin, PR = progesterone receptor, TNBC = triple negative breast cancer.

respectively) (Tables 1 and 2). Furthermore, when the analysis was done for the entire study cohort, we found a significant association between NS expression and p53 status ($P = .037$) (Table 3).

This was further confirmed using Curtis breast cancer database, which also revealed that GNL3 gene expression was higher in the cases associated with mutated p53 compared to wild type p53 (Fig. 2 D). This proves that in breast cancer, higher NS expression is associated with p53 mutation and inactivation, which might explain its role in cell cycle progression and cell survival.

4.6. GNL3 gene expression to be associated with poor patients' outcome represented as shorter distance metastasis survival

For better evaluation of the prognostic role of NS in breast cancer, we next appraised the association between GNL3 gene expression levels and patients' outcome with relapse-free survival

(RFS) as an end point in 1881 breast cancer patient's samples using GOBO database.

Indeed, the results disclosed that high GNL3 gene expression levels are significantly associated with poor patient outcome presented as shorter relapse free survival (RFS) ($P = .00034$) (Fig. 3A).

Moreover, classification of those patients according to their tumors' differentiation levels, revealed that while, GNL3 gene expression levels had no effect on patients' outcome in the well-differentiated tumors, it showed a significant association with poor patients' outcome in the less-differentiated moderate and poorly differentiated tumors ($P = .00017$, $P = .04371$ respectively) (Fig. 3B).

In addition, GNL3 gene expression levels showed a significant association with patient outcome in the early LN negative tumors with no effect on the outcome of LN positive tumors. This indicates that NS might play an important role in early breast cancer tumorogenesis and disease progression (Fig. 3C).
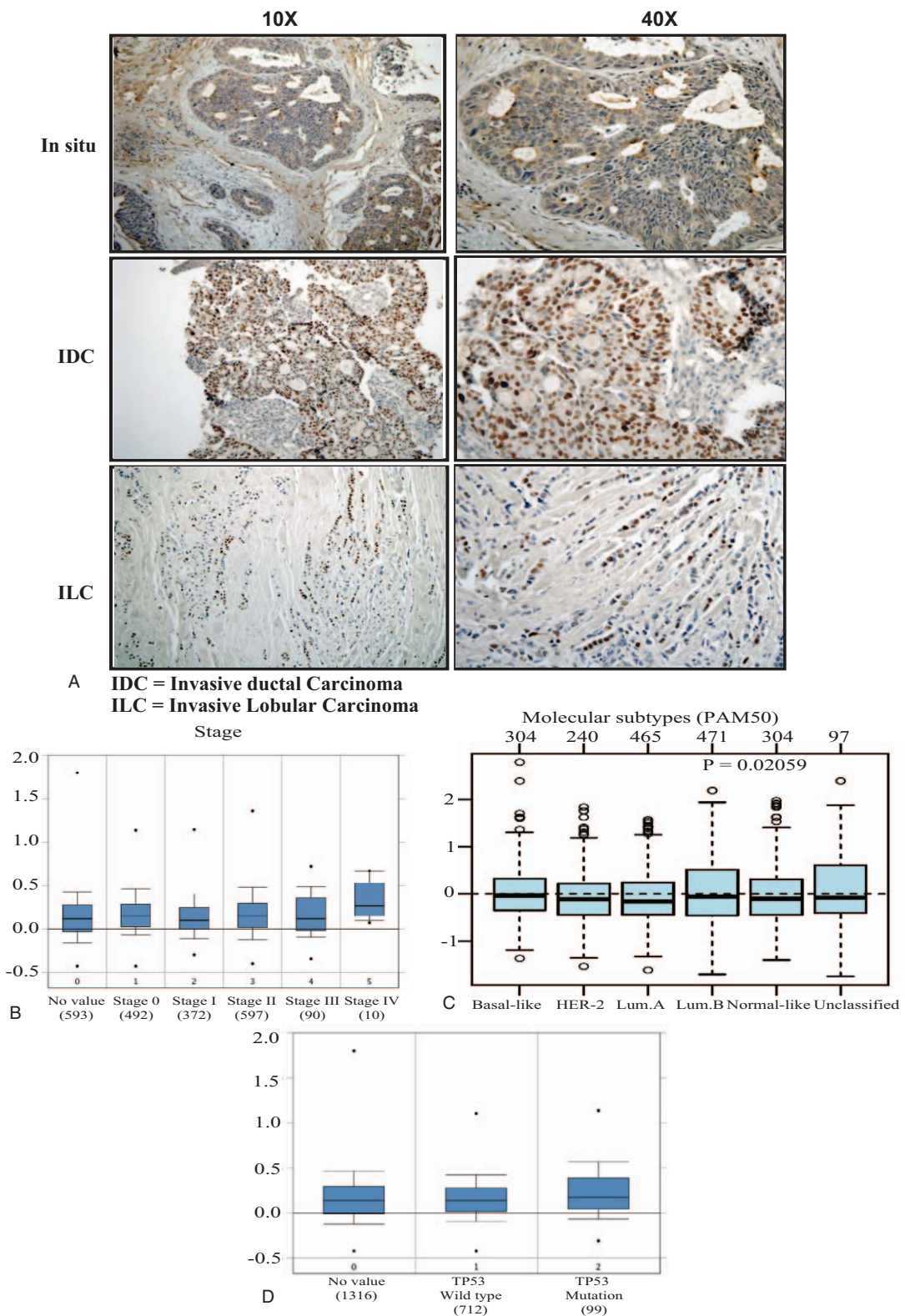
Figure 2. The association between NS expression and tumor stage, molecular subtype and P53 status. (A) Representative pictures of immunohistochemical expression of NS in breast cancer samples from early in situ carcinoma and more advanced invasive ductal and lobular carcinoma. (B) GNL3 gene expression and its association with tumor stage in more than 2000 breast cancer samples obtained from Curtis dataset of ONCOMINE database. (C) GNL3 gene expression and its association with breast cancer molecular subtypes classified according to PAM50 in 1881 breast cancer samples obtained from GOBO database. (D) GNL3 gene expression and its association with P53 status in more than 2000 breast cancer samples obtained from Curtis dataset of ONCOMINE database. GOBO = gene expression based outcome, NS = nucleostemin.
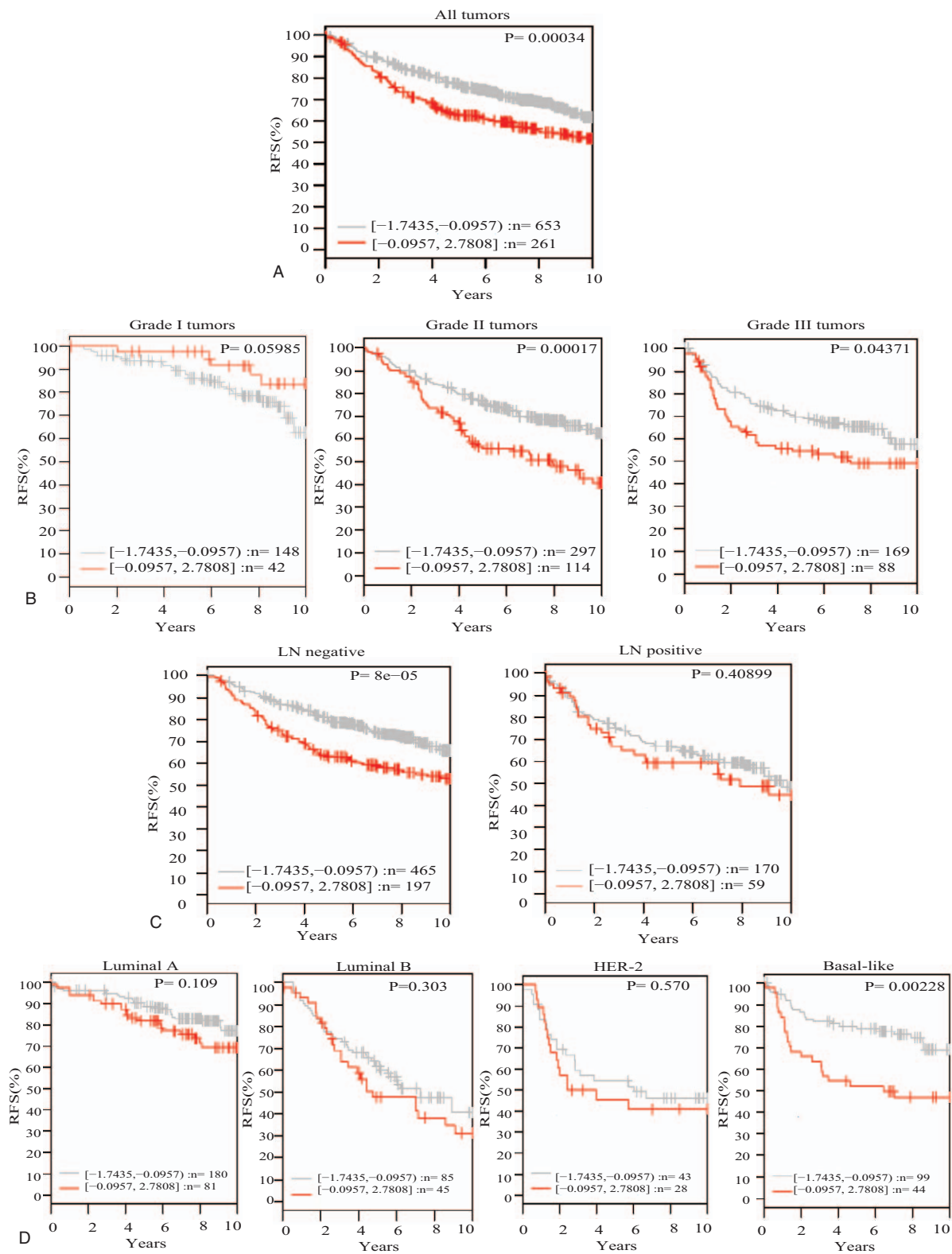
Figure 3. GNL3 gene expression and its association with patient outcome represented as relapse free survival in 1881 breast cancer samples obtained from GOBO database. (A) GNL3 gene expression and its association with patient outcome in the whole patients sample. (B) GNL3 gene expression and its association with patient outcome in different tumor grades. (C) GNL3 gene expression and its association with patient outcome in LN negative and positive samples. (D) GNL3 gene expression and its association with patient outcome in different breast cancer subtypes. GOBO = gene expression based outcome, LN = lymph node, NS = nucleostemin.

Finally, and owing to the essential and emerging role of breast cancer molecular subtypes in determining patients' outcome and therapeutic options for breast cancer, next we reviewed the association between GNL3 gene expression levels and patients' outcome in different breast cancer subtypes. Interestingly, GNL3 gene expression showed no significant association with patients' outcome in luminal A, B, and HER-2, though it showed significant association with poor patients' outcome in the highly aggressive, stem cells- enriched, basal-like breast cancer tumors (Fig. 3D).

5. Discussion

Breast CSCs are believed to play a crucial role in breast cancer aggressiveness, in addition to their association with tumor recurrence and drug resistance.[3] Consequently, members of pathways involved in breast cancer stemness might provide ideal candidates to predict patients with higher risk of poor outcome. Earlier research proved that NS is implicated in regulating stem cell maintenance and proliferation in different types of cancer cells.[25] However, its role in the prognosis and prediction of patient outcome in breast cancer patient still needs to be further elucidated.

In this context, we report in the present study, the prognostic value of NS expression in a multiethnic cohort of breast cancer patients from UAE compared to a cohort of commercial TMA of breast cancer. Interestingly, our study showed a variable NS expression among the 2 cohorts. Indeed, UAE cohort showed lower NS expression compared to the commercial TMA, which could be attributed to the difference of clinicopathological features of both cohorts. Particularly, the UAE cohort included predominantly stage I and II tumors, whereas the commercial TMA cohort showed more diversity in the tumors' stage with around one-third being of the more advanced stage III and IV.

Importantly, we should mention that our report has certain limitations. One of the main limitations we observe is the limited sample size of the UAE patient's cohort, in addition to the limited clinicopathological as well as follow up information available for those patients. This resulted in insignificant association between NS expression and all of the clinic-pathological parameters in the UAE cohort. This limitation associated with sample size is not restricted to our report, but it is also observed in large group of histopathological studies. This is attributed to the fact that many of these reports are retrospective and based upon availability of cases and was not based on sample size calculations essential for primary research question evaluation.[26] However, and to overcome this limitation, we investigate the NS expression in additional cohort (commercial TMA purchased from Pantomics), which contains another 102 cases. Moreover, we thoroughly reviewed and analyzed 2 publicly available databases to explore the correlation of NS expression and the well-known prognostic parameters in around 2000 breast cancer samples. The results from both databases endorsed our results and provided compelling evidence that NS expression in breast cancer samples is associated with loss of tumor differentiation, advanced tumor stage and tumor progression.

The association between high NS expression and more aggressive phenotype was observed in other reports. Indeed, Yoshida et al, 2014[27] reported NS, to be a marker of advanced malignant phenotype in oral squamous cell carcinoma. In addition, and consistent with earlier studies,[27,28] our results also revealed that NS expression is increased with tumor progression presented as larger tumor size, LN metastasis, and advanced tumor stage. However, Kobayashi et al 2014 [12] showed that NS had no significant association with tumor size, LN involvement as well as distant metastasis.

Another noteworthy finding of the present study was the detection of higher NS immunorecativity in the more aggressive molecular subtypes including HER-2 and TNBC, the latter being usually associated with worse prognosis and characterized by high-grade tumors[29] and enriched with stemness and epithelial to mesenchymal transition-related genes.[30] In support of our results, Lin et al 2010 concluded that in mammary tumors, basal cell type expressed more NS than the luminal cell type. Furthermore, NS-enriched mammary tumor cells displayed stronger in vitro and in vivo tumorigenic activities.[31]

Remarkably, our results revealed a significant association between positive NS expression and positive p53 expression, which usually denotes p53 mutation and inactivation. This is in agreement with Kobayashi et al 2014[12] who showed a similar association and demonstrated that tumors characterized by higher levels of co-expression of NS and p53 are associated with worse prognosis presented as disease-free survival (DFS).

In his review, Tsai 2014, discussed the possible interplay between NS and p53 and stated that in cells with wild-type p53, NS depletion turns on p53 and prompt cell cycle arrest, however, this might be completely or partially reversed upon p53 knockdown.[32–34] Furthermore, the fact that NS interacts with MDM2 to modulate the activity of p53 was proved by many authors.[23,24] On the other hand, some authors showed accelerated death of NS-knockout MEF cells upon loss of p53[35] inferring that p53 is not essential for NS function in cell proliferation maintenance.

Finally, we analyzed data of 1881 breast cancer patients to probe the association between NS gene (GNL3) expression levels and patients' outcome presented as relapse-free survival (RFS), which is an important indicator of tumor recurrence. The results confirmed a significant association between GNL3 expression and poor patients' outcome presented as shorted RFS. This was more evident in the more aggressive grade II and III as well as basal-like breast cancer subtype. This goes with other reports that also showed NS expression to be a marker of tumor recurrence[28] and poor patient outcome.[12]

In conclusion, our results disclosed that NS protein, as well as gene expression levels, can be used as markers of tumor progression, aggressive histological phenotype as well as higher risk of tumor recurrence in breast cancer, implying that NS may be a potential target for CSC-associated breast cancer management.

Author contributions

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