THE CLINICOPATHOLOGICAL SIGNIFICANCE OF LAMIN A/C, LAMIN B1 AND LAMIN B RECEPTOR mRNA EXPRESSION IN HUMAN BREAST CANCER

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Abstract: Lamin A/C (LMNA), lamin B1 (LMNB1) and lamin B receptor (LBR) have key roles in nuclear structural integrity and chromosomal stability. In this study, we have studied the relationships between the mRNA expressions of A-type lamins, LMNB1 and LBR and the clinicopathological parameters in human breast cancer. Samples of breast cancer tissues (n = 115) and associated non-cancerous tissue (ANCT; n = 30) were assessed using reverse transcription and quantitative PCR. Transcript levels were correlated with clinicopathological data. Higher levels of A-type lamins and LMNB1 mRNA expression were seen in ANCT. Higher lamin A/C expression was associated with the early clinical stage (TNM1 vs. TNM3 – 13 vs. 0.21; p = 0.0515), with better clinical outcomes (disease-free survival vs. mortality – 11 vs. 1; p = 0.0326), and with better overall (p = 0.004) and disease-free survival (p = 0.062). The expression of

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Abbreviations used: C1 – confidence interval; CK19 – cytokeratin 19; DF – disease-free survival; DR – distant disease recurrence; LBR:ANCT – associated non-cancerous tissue, lamin B receptor; LMDM – lamin B deficient micro-domains; LR – local disease recurrence; mTOR – mammalian target of rapamycin; NPI – Nottingham Prognostic Index; qPCR – quantitative polymerase chain reaction; TNM – clinical stage according to tumor size, nodal status and presence of distant metastases
LMNB1 declined with worsening clinical outcome (disease-free vs. mortalities – 0.0011 vs. 0.000; p = 0.0177). LBR mRNA expression was directly associated with tumor grade (grade 1 vs. grade 3 – 0.00 vs. 0.00; p = 0.0479) and Nottingham Prognostic Index (NPI1 vs. NPI3 – 0.00 vs. 0.00; p = 0.0551). To the best of our knowledge, this is the first study to suggest such a role for A-type lamins, lamin B1 and LBR in human breast cancer, identifying an important area for further research.

**Key words:** Lamin A/C, Lamin B, Lamin B receptor, Breast cancer, qPCR, Chromosomal instability, Cell senescence, Cell cycle, DNA repair, Ageing

**INTRODUCTION**

Lamin A, lamin B and lamin B receptor (LBR), are nuclear proteins that are found on the inner side of the nuclear envelope. Nuclear lamins A, B and C make up the nuclear lamina, interacting with many integral membrane proteins of the inner nuclear membrane and with proteins associated with chromatin. LBR is an integral membrane protein that helps anchor B-type lamins to the nuclear membrane. It also binds HP1, a chromatin-binding protein associated with heterochromatin [1]. Lamins, especially lamin B, as part of the nuclear lamina, anchor specific areas of the genome to the nuclear periphery [2] and are involved in chromosome positioning [3]. These are often gene-poor regions of the genome, helping to the functionally organize the cell’s chromosomes. Lamins can also be found deep within the nucleoplasm [4, 5], where they may have roles in DNA replication, transcription, mRNA splicing and DNA repair [1]. Lamin A and LBR are both involved in cellular differentiation, but inversely with lamin A promoting it and LBR preventing it [6]. Mutations in the LMNA gene cause a spectrum of degenerative disorders ranging from muscular dystrophies to premature ageing known as the laminopathies [4, 7-9]. A-type lamins have been implicated in prostate, colon and gastric carcinogenesis, while B-type lamins have been suggested to have roles in prostate cancer and hepatocarcinoma [10-12]. In this study, we have endeavored to elucidate the relationships between the mRNA expressions of A-type lamins and LMNB1 and LBR genes and the clinicopathological parameters of human breast cancer.

**MATERIALS AND METHODS**

**Samples**

Tissue samples were collected from patients with their informed consent and with ethical approval as per contemporaneous institutional guidelines. Immediately after surgical excision, a tumor sample was taken from the tumor area, and a second sample was taken from the associated non-cancerous tissue (ANCT) within 2 cm of the tumor, without affecting the assessment of tumor margins. Breast cancer tissues (n = 115) and normal background tissues (n = 30)
were collected and stored at -80ºC in liquid nitrogen until the commencement of 
this study. This patient cohort has been the subject of a number of completed and 
on-going studies [13-15]. The cohort was reflective of the patient population it 
was drawn from in terms of the proportions of patient categories based on clinical 
stage, histopathology, Nottingham Prognostics Index (NPI), and clinical outcome.

Table 1. Clinicopathological data describing the patient cohort.

| Parameter                | Category                        | Number |
|--------------------------|---------------------------------|--------|
| Node status              | Node positive                   | 53     |
|                          | Node negative                   | 62     |
| Tumor grade              | 1                               | 20     |
|                          | 2                               | 39     |
|                          | 3                               | 54     |
| Nottingham Prognostic Index | 1                          | 58     |
|                          | 2                               | 38     |
|                          | 3                               | 15     |
| Tumor type               | Ductal                          | 89     |
|                          | Lobular                         | 12     |
|                          | Medullary                       | 2      |
|                          | Tubular                         | 1      |
|                          | Mucinous                        | 4      |
|                          | Other                           | 7      |
| TNM staging              | 1                               | 61     |
|                          | 2                               | 37     |
|                          | 3                               | 7      |
|                          | 4                               | 4      |
| Receptor Status          | Estrogen positive (ER+)          | 35     |
|                          | Estrogen negative (ER-)          | 69     |
|                          | Human epidermal growth factor receptor 2 positive (Her2/Neu +) | 24 |
|                          | Human epidermal growth factor receptor 2 negative (Her2/Neu -) | 83 |
| Clinical outcome         | Disease-free                    | 81     |
|                          | With local recurrence           | 7      |

All the patients were treated according to local guidelines, following discussions 
in multidisciplinary meetings. Patients undergoing breast-conserving surgery 
also underwent radiotherapy. Hormone-sensitive patients were given tamoxifen. 
Hormone-insensitive cases, high-grade cancer and node-positive cases were 
treated with adjuvant therapy. At the time of collection of the samples, neo-
adjuvant therapy was yet to be incorporated into local treatment guidelines. At 
the time of biopsy, the patients would not have undergone any chemotherapy or 
radiotherapy. Therefore, it should be emphasized that the readings seen in this
cohort are more likely to be in keeping with the natural history of the pathology. Clinicopathological data (Table 1) was collected from the patient charts, and was collated in an encrypted database. It should be stressed that the use of a long-standing albeit well curated cohort comes with several caveats. Firstly, the clinical and statistical database is stored and maintained by program suites and custom scripts that have since become legacy, with long-entrenched settings. Outputs are limited to four decimal places, and smaller, more exact readings could not be extracted without a risky and disruptive porting of the database and its associated scripts and settings to an unfamiliar alternative. This very rarely may result in a situation in which we may be informed by the p value generated that the difference between the compared values is significant, even though the actual values would be too minute to be displayed by the statistical analysis output. Furthermore, it has to be reiterated that this was a cohort randomly selected from a tissue library with patient categories reflective of the general patient population. However, over the years, the reserves of RNA and cDNA of some cases originally collected have been exhausted, and thus may not be available for analysis. Consequently, the numbers within certain patient categories may be marginal. However, the results from this cohort as a whole achieved statistical significance as detailed in the following sections.

RNA extraction kits and reverse transcription kits were obtained from AbGene Ltd. (Epsom, Surrey, UK). PCR primers were designed using Beacon Designer (Palo Alto, CA, USA) and synthesized by Invitrogen Ltd. (Paisley, United Kingdom). Custom made hot-start master mix for quantitative PCR was from AbGene [16].

Tissue processing, RNA extraction and cDNA synthesis
Approximately 10 mg of cancerous tissue was homogenized. A larger amount of ANCT (20–50 mg) was used because its high fat content made it difficult to obtain sufficient RNA for analysis. The concentration of RNA was determined using a UV spectrophotometer (Wolf Laboratories, York, UK) to ensure adequate amounts of RNA for analysis. Reverse transcription was carried out using a reverse transcription kit (AbGene) with an anchored olig (dT) primer using 1 mg of total RNA in a 96-well plate to produce cDNA. The quality of cDNA was verified using β-actin primers (primers 5’-ATGATA-TCGCGCGCTCGTC-3’ and 5’-CGCTCCTGAGGATCTTTCA-3’) [16].

Quantitative analysis
Transcripts of the cDNA library were determined using real-time quantitative PCR based on Amplifluor technology. The PCR primers were designed using Beacon Designer software (Premier Biosoft International Ltd., Palo Alto, CA, USA), but an additional sequence, known as the Z sequence (5’-ACTGAA-CCTGACCCTACA-3’), which is complementary to the universal Z probe (Invitrogen Inc., Oxford, UK), was added to the primer (Table 2). During primer design, it became apparent that developing primers specific to lamin A or C was
technically difficult if not infeasible. Consequently, the primer used was directed non-specifically towards A-type lamins.

Table 2. Primers used in the study.

| Gene                  | Sequence (5’–3’)                  |
|-----------------------|-----------------------------------|
| Lamin A/C forward     | AAGCTTCGAGACCTGGAG                |
| Lamin A/C Z reverse   | ACTGAAACCTGACGCTCAATCTCCCGCTCTTTTC|
| Lamin B1 forward      | ATCGAGCTGGGCAGT                  |
| Lamin B1 Z reverse    | ACTGAAACCTGACGCTCAATCTCCCGCTCTTG|
| Lamin B receptor forward | TGGGTGATCTCATCTGG            |
| Lamin B receptor Z reverse | ACTGAAACCTGACGCTCAATCTCCCGCTCTTG |
| CK19 forward          | CAGGCTCGAGCTTACTGAC             |
| CK19 Z reverse        | ACTGAAACCTGACGCTCAATCTCCCGCTCTTG|

The reaction was carried out under the following conditions: 94°C for 12 min and 50 cycles of 94°C for 15 s, 55°C for 40 s, and 72°C for 20 s. The levels of each transcript were generated from a standard that was simultaneously amplified within the samples. Levels of expression were normalized against cytokeratin 19 (CK19). With every run of the PCR, a negative and positive control was employed, using a known cDNA sequence (podoplanin) [16].

Statistical analysis
Analysis of the data was performed using the Minitab 12 statistical software package (Minitab Ltd., Coventry, UK.) using a custom-written macro (Stat06e.mtb). Medians were compared using the Mann-Whitney U-test, while means were compared using the two-sample t-test. The transcript levels within the breast cancer specimens were compared to those of the ANCT and correlated with clinicopathological data collected over a 10-year follow-up period. After determining the underlying distribution, non-parametric tests were deemed to be more appropriate for this cohort.

P values less than 0.05 were considered significant, whereas p values between 0.05 and 0.10 were considered marginally significant. For purposes of the Kaplan-Meier survival analysis, the samples were divided arbitrarily into high and low transcription groups, with the mean copy number for the moderate prognostic group as defined by NPI serving as the dividing line. Survival analyses were performed using PSAW18 (SPSS Inc., Chicago, IL, USA).

RESULTS

Higher levels of A-type lamin mRNA expression were seen in associated non-cancerous tissue (ANCT vs. cancerous tissue – 65 vs. 5; p = 0.0006). Furthermore, A-type lamins expression was found to be inversely associated with clinical stage (TNM1 vs. 3 – 13 vs. 0.21; p = 0.0515). Decreased LMNA
mRNA expression was also associated with adverse clinical outcomes (disease-free survival vs. mortality – 11 vs. 1; \( p = 0.0326 \); Tables 3 and 4). The differences between categories based on receptor expression did not achieve statistical significance.

Kaplan-Meier analysis suggested that higher \( LMNA \) expression had a highly significant association with better overall survival \( (p = 0.004) \), and a moderately significant association with better disease-free survival \( (p = 0.062; \text{Figs } 1 \text{ and } 2) \). \( LMNB1 \) expression was found to be higher in ANCT than in cancerous tissue \( (ANCT \text{ vs. cancerous tissue } – 0.12 \text{ vs. } 0.00; p < 0.0001) \). This difference remained highly significant in all patient categories by tumor grade, clinical stage and Nottingham Prognostic index. The differences between categories based on receptor expression did not achieve statistical significance.

Table 3. Comparison of mRNA expression levels of A-type lamins (A/C) in subgroups within the cohort.

| Patient and tumor characteristics | Median(s) | 95% CI | P value |
|----------------------------------|-----------|--------|---------|
| Tumor grade                      |           |        |         |
| 1 vs. 2                          | 11.6 vs. 5.5 | -30.2, 7.1 | 0.7855 |
| 1 vs. 3                          | 11.6 vs. 3  | -3, 11  | 0.6092  |
| 2 vs. 3                          | 5.5 vs. 3   | -1, 17  | 0.4572  |
| NPI                              |           |        |         |
| 1 vs. 2                          | 6 vs.10.4  | -2, 19  | 0.4583  |
| 1 vs. 3                          | 6 vs. 1.3   | -2, 40  | 0.4050  |
| 2 vs. 3                          | 10.4 vs. 1.3| -2.8, 12| 0.8281  |
| TNM                              |           |        |         |
| 1 vs. 2                          | 13 vs. 1.3  | 0, 20   | 0.1194  |
| 1 vs. 3                          | 13 vs. 0.21 | 2, 151  | 0.0515  |
| 1 vs. 4                          | 13 vs. 9.94| -19, 452| 0.4694  |
| 2 vs. 3                          | 1.3 vs. 0.21| -0.2, 29.2| 0.3045 |
| 2 vs. 4                          | 1.3 vs. 9.94| -19.7, 111.3| 0.9825 |
| 3 vs. 4                          | 0.21 vs. 9.94| -60.03, 2.58| 0.5083 |
| Survival                         |           |        |         |
| DF vs. LR                        | 11 vs. 0   | -121, 44 | 0.4686  |
| DF vs. DR                        | 11 vs. 1   | -0, 163 | 0.1423  |
| DF vs. D                         | 11 vs. 1   | -1, 39  | 0.0326  |
| DF vs. LR/DR/D                   | 11 vs. 1   | -0, 20  | 0.0162  |

CI – confidence interval, DF – disease-free survival, LR – local disease recurrence, DR – distant disease recurrence, D – death from breast cancer, NPI – Nottingham Prognostic Index, TNM – clinical stage according to tumor size, nodal status and presence of distant metastases
Table 4. mRNA expression levels of A-type lamins (A/C) in subgroups within the cohort.

| Patient and tumor characteristics | Median | Trimmed mean | Interquartile range (Q1-Q3) |
|----------------------------------|--------|--------------|----------------------------|
| Tumor grade                      |        |              |                            |
| 1                                | 11.6   | 88           | 0-54                       |
| 2                                | 5.5    | 1000         | 0-454                      |
| 3                                | 3      | 89           | 0-50                       |
| NPI                              |        |              |                            |
| 1                                | 6      | 510          | 0-179                      |
| 2                                | 10.4   | 104          | 0-30                       |
| 3                                | 1.3    | 25           | 0-44                       |
| TNM                              |        |              |                            |
| 1                                | 13     | 522          | 0-181                      |
| 2                                | 1.3    | 95           | 0-37                       |
| 3                                | 0.21   | 4.84         | 0-2.61                     |
| 4                                | 9.94   | 20           | 0-50.1                     |
| Survival                         |        |              |                            |
| DF                               | 11     | 345          | 0-157                      |
| LR                               | 0      | 209          | 0-183                      |
| DR                               | 1.28   | 5.60         | 0.02-13.33                 |
| D                                | 1.06   | 5.36         | 0.01-9.44                  |
| LR/DR/D                          | 1.0    | 19.1         | 0.0-22.0                   |

DF – disease-free survival, LR – local disease recurrence, DR – distant disease recurrence, D – death from breast cancer, NPI – Nottingham Prognostic Index, TNM – clinical stage according to tumor size, nodal status and presence of distant metastases; *Range (minimum to maximum).

Fig. 1. Disease-free survival curve based on mRNA expression of LMNA. Curve A (lower transcription group) and curve B (higher transcription group) are defined by the median of the moderate risk group by the Nottingham Prognosis Index (NPI2) serving as the dividing line.
Fig. 2. Overall survival curve based on the mRNA expression of LMNA. Curve A (lower transcription group) and curve B (higher transcription group) are defined by the median of the moderate risk group by the Nottingham Prognosis Index (NPI2) serving as the dividing line.

Table 5. Comparison of lamin B1 mRNA expression levels in subgroups within the cohort.

| Patient and tumor characteristics | Median(s) | 95% CI       | P value |
|----------------------------------|-----------|--------------|---------|
| **Tumor grade**                  |           |              |         |
| 1 vs. 2                          | 0.000 vs. 0.001 | -0.013, -0.000 | 0.4469  |
| 1 vs. 3                          | 0.000 vs. 0.001 | -0.004, 0.000  | 0.8313  |
| 2 vs. 3                          | 0.000 vs. 0.000 | -0.000, 0.002  | 0.5081  |
| **NPI**                          |           |              |         |
| 1 vs. 2                          | 0.009 vs. 0.001 | -0.001, 0.013  | 0.1139  |
| 1 vs. 3                          | 0.009 vs. 0.000 | -0.001, 0.052  | 0.1722  |
| 2 vs. 3                          | 0.001 vs. 0.000 | -0.000, 0.001  | 0.8281  |
| **TNM**                          |           |              |         |
| 1 vs. 2                          | 0.006 vs. 0.001 | 0.000, 0.011   | 0.2196  |
| 1 vs. 3                          | 0.006 vs. 0.001 | -0.001, 0.101  | 0.6570  |
| 1 vs. 4                          | 0.006 vs. 0.000 | -0.000, 0.528  | 0.3191  |
| 2 vs. 3                          | 0.0008 vs. 0.0005 | -0.0012, 0.0051 | 0.8852  |
| 2 vs. 4                          | 0.0008 vs. 0.0001 | -0.03530, 0.0299 | 0.4959  |
| 3 vs. 4                          | 0.0005 vs. 0.0001 | -0.3746, 0.2029 | 0.7055  |
| **Survival**                     |           |              |         |
| DF vs. LR                        | 0.0011 vs. 0.3000 | -0.47, 0.00    | 0.4094  |
| DF vs. DR                        | 0.0011 vs. 0.0040 | -0.013, 0.064  | 0.7467  |
| DF vs. D                         | 0.0011 vs. 0.0000 | 0.0001, 0.0146 | 0.0177  |
| DF vs. LR/DR/D                   | 0.0011 vs. 0.0000 | 0.000, 0.004   | 0.284   |

CI – confidence interval, DF – disease-free survival, LR – local disease recurrence, DR – distant disease recurrence, D – death from breast cancer, NPI – Nottingham Prognostic Index, TNM – clinical stage according to tumor size, nodal status and presence of distant metastases
Table 6. mRNA expression levels of lamin B1 in subgroups within the cohort.

| Patient and tumor characteristics | Median | Trimmed mean | Interquartile range (Q1-Q3) |
|----------------------------------|--------|--------------|-----------------------------|
| **Tumor grade**                  |        |              |                             |
| 1                                | 0.00   | 0.41         | 0.00-0.01                   |
| 2                                | 0.00   | 1.25         | 0.00-0.14                   |
| 3                                | 0.00   | 0.1          | 0.0-0.1                    |
| **NPI**                          |        |              |                             |
| 1                                | 0.0    | 1.4          | 0.0-0.3                    |
| 2                                | 0.001  | 0.326        | 0.000-0.014                |
| 3                                | 0.0003 | 0.0406       | 0.0000-0.0594              |
| **TNM**                          |        |              |                             |
| 1                                | 0.0    | 1.0          | 0.0-0.2                    |
| 2                                | 0.00   | 0.27         | 0.00-0.02                  |
| 3                                | 0.0005 | 0.0599       | 0.0001-0.2028              |
| 4                                | 0.0001 | 0.0938       | 0.0000-0.2814              |
| **Survival**                     |        |              |                             |
| DF                               | 0.0    | 0.6          | 0.0-0.1                    |
| LR                               | 0.3    | 4.09         | 0.00-9.36                  |
| DR                               | 0.0    | 1.27         | 0.00-3.17                  |
| D                                | 0.0    | 0.0048       | 0.0000-0.0017              |
| LR/DR/D                          | 0.0    | 0.732        | 0.0000-0.113               |

CI – confidence interval, DF – disease-free survival, LR – local disease recurrence, DR – distant disease recurrence, D – death from breast cancer, NPI – Nottingham Prognostic Index, TNM – clinical stage according to tumor size, nodal status and presence of distant metastases

In addition, the expression of LMNB1 declined with worsening clinical outcome. This association attains statistical significance when comparing patients with disease-free survival with disease-related mortalities (disease-free vs. mortalities – 0.0011 vs. 0.00; p = 0.0177; Tables 5 and 6). However, Kaplan-Meier analysis comparing high and low transcription groups for LMNB1 expression failed to show a statistically significant association with survival. Furthermore, less salient yet statistically significant findings were seen when studying LBR mRNA expression. Specifically, direct association with tumor grade (grade 1 vs. grade 3 – 0.00 vs. 0.00; p = 0.0479) and the Nottingham Prognostic Index (NPI1 vs. NPI3 – 0.00 vs. 0.00; p = 0.0551) were observed (Tables 7 and 8). However, Kaplan-Meier analysis comparing high and low transcription groups for LMNB1 expression failed to show a statistically significant association with survival. Furthermore, the differences between categories based on receptor expression did not achieve statistical significance.
Table 7. Comparison of LBR mRNA expression levels in subgroups within the cohort.

| Patient and tumor characteristics | Median(s) | 95% CI     | p-Value |
|-----------------------------------|-----------|------------|---------|
| Tumor grade                       |           |            |         |
| 1 vs. 2                           | 0.0 vs. 0.0 | 0.1, -0.0  | 0.4096  |
| 1 vs. 3                           | 0.0 vs. 0.0 | -21.3, -0.0 | 0.0479  |
| 2 vs. 3                           | 0.0 vs. 0.0 | -0.1, 0.0   | 0.1158  |
| NPI                               |           |            |         |
| 1 vs. 2                           | 0.0 vs. 0.0 | 0.1, 0.1   | 0.5121  |
| 1 vs. 3                           | 0.0 vs. 0.0 | -448.9, 0.2 | 0.0551  |
| 2 vs. 3                           | 0.0 vs. 0.0 | -448.1, 0.1 | 0.1794  |
| TNM                               |           |            |         |
| 1 vs. 2                           | 0.0 vs. 0.0 | 0.2, -0.1   | 0.2686  |
| 1 vs. 3                           | 0.0 vs. 0.0 | -2.6, -0.0  | 0.2758  |
| 1 vs. 4                           | 0.0 vs. 0.0 | -111.2, 10.8  | 0.7954  |
| 2 vs. 3                           | 0.0 vs. 0.0 | -2.5, 321.8  | 0.7241  |
| 2 vs. 4                           | 0.0 vs. 0.0 | -0.0, 607.9  | 0.8433  |
| 3 vs. 4                           | 0.0 vs. 0.0 | 0.6366      |         |
| Survival                          |           |            |         |
| DF vs. LR                         | 0.0 vs. 0.0 | -0.0, 114.2 | 0.4138  |
| DF vs. DR                         | 0.0 vs. 0.0 | 0.0, 0.0    | 1.0000  |
| DF vs. D                          | 0.0 vs. 0.0 | -0.1, 0.2   | 0.9916  |
| DF vs. LR/DR/D                    | 0.0 vs. 0.0 | 0.0, -0.0   | 0.2696  |

CI – confidence interval, DF – disease-free survival, LR – local disease recurrence, DR – distant disease recurrence, D – death from breast cancer, NPI – Nottingham Prognostic Index, TNM – clinical stage according to tumor size, nodal status and presence of distant metastases.

Table 8. mRNA expression levels of LBR in subgroups within the cohort.

| Patient and tumor characteristics | Median | Trimmed mean | Interquartile range (Q1-Q3) |
|-----------------------------------|--------|--------------|----------------------------|
| Tumor grade                       |        |              |                            |
| 1                                 | 0.0    | 6.7          | 0.0-0.9                    |
| 2                                 | 0.0    | 112.2        | 0.0-121                    |
| 3                                 | 0.0    | 370          | 0.0-508                    |
| NPI                               |        |              |                            |
| 1                                 | 0.0    | 149          | 0.0-24.2                   |
| 2                                 | 0.0    | 133          | 0.0-47                     |
| 3                                 | 0.0    | 406          | 0.0-564                    |
| TNM                               |        |              |                            |
| 1                                 | 0.0    | 121.9        | 0.0-6.6                    |
| 2                                 | 0.0    | 371          | 0.0-527                    |
| Patient and tumor characteristics | Median | Trimmed mean | Interquartile range (Q1-Q3) |
|----------------------------------|--------|--------------|---------------------------|
| 3                                | 0.3    | 96.9         | 0.0-32.6                  |
| 4                                | 0.0    | 69.6         | 0.0-208.8                 |
| Survival                         |        |              |                           |
| DF                               | 0.0    | 191          | 0.0-201                   |
| LR                               | 0.0    | 140          | 0.0-3                     |
| DR                               | 0.0    | 0.00         | 0.0-0.0                   |
| D                                | 0.0    | 318          | 0.0-238                   |
| LR/DR/D                          | 0.0    | 200          | 0.0-0.0                   |

DF – disease-free survival, LR – local disease recurrence, DR – distant disease recurrence, D – death from breast cancer, NPI – Nottingham Prognostic Index, TNM – clinical stage according to tumor size, nodal status and presence of distant metastases

**DISCUSSION**

Nuclear envelope proteins have important functions in cell cycle regulation, cell differentiation, functional genome organization, gene expression and processing, DNA repair and intracellular signaling and are also probably involved in cellular senescence and ageing. They can be categorized into three groups: nuclear pore proteins, which mediate transit of materials across the nuclear envelope; nuclear lamina proteins, which constitute the nuclear lamina underneath the nuclear membrane; and integral membrane proteins, which are embedded in the nuclear membranes. Many of these proteins are evolutionarily conserved among vertebrates [8, 9] and have significant homology with proteins in simpler non-vertebrate organisms.

Seven nuclear lamina proteins have been identified, and have been studied significantly in both humans and murine models [8]. The main lamins are lamin A and C which are transcribed from a single gene designated *LMNA* (1q21.2-q21.3) using alternative splicing [17]. The gene was identified in the 1980s. In 1993, *LMNA* was first found to be involved in the pathogenesis of Emery-Dreifuss muscular dystrophy [18]. Since then, mutations in *LMNA* have been found to be implicated in a spectrum of degenerative syndromes causing skeletal and cardiac myopathies, lipodystrophies, diabetes and neuropathies [9]. Furthermore, mutations in *LMNA* and some of its binding proteins at the nuclear envelope have been implicated in Hutchinson-Gilford Progeria Syndrome (HGPS). These conditions have been collectively termed ‘laminopathies’, and have been studied extensively in order to better understand the different diseases and the underlying pathways that lamin A/C are involved in [19]. Recent studies in murine models have suggested that the defective lamin A/C may mediate its effects through the mammalian target of rapamycin complex 1 (mTORC1) pathway. Indeed rapamycin improves the appearance, chromatin organization and proliferative life-span of HGPS cells in culture presumably by degrading the accumulated toxic lamin A protein progerin [20, 21]. Furthermore, Ramos *et al.*
has found that rapamycin could reverse pathological changes in \textit{Lmna} deficient mice \cite{22}.

B-type lamins have up to three known isotypes. Lamin B1 is encoded by the gene \textit{LMNB1}, which localizes to chromosome 5q23.3-q31.1 \cite{23}. B-type lamins are believed to have roles in cellular proliferation and senescence \cite{24, 25} and brain development \cite{26}. Defects in B-type lamin expression and transcription have been implicated in a number of genetic diseases. Over-expression due duplication of lamin B1 has been implicated in the pathogenesis of adult-onset autosomal dominant leukodystrophy, which resembles multiple sclerosis in its symptomatology \cite{27}. Similarly, certain variants of lamin B2 have been implicated in an acquired sporadic form of leukodystrophy referred to as Barraquer-Simons syndrome \cite{28}.

Lamin B is known to interact with the lamin B receptor (LBR), which is an integral nuclear membrane protein embedded in the inner nuclear membrane. LBR also interacts with heterochromatin, and is believed to have a key role in the normal distribution of chromatin within the post-mitotic nucleus. In addition to lamin proteins and DNA, LBR has large number of downstream effectors, believed to impact the cell cycle \cite{29} and has a negative role in cellular differentiation \cite{6}. Defects in LBR have been implicated in a hematological condition known as the Pelger-Huët anomaly. Heterozygous cases of this condition have bi-lobed rather than multi-lobed neutrophils. Homozygous embryos fail to reach term \cite{30}.

The LBR moiety is also known to incorporate a C17 sterol reductase domain. This function was discovered whilst studying non-viable human embryos suffering from a congenital anomaly called HEM/Greenberg dysplasia, which is characterized by defects in cholesterol metabolism, skeletal defects, and in utero lethality \cite{31}.

More recently, the A- and B-type lamins have increasingly been found to have roles in various types of cancer \cite{32} and LBR has been found lacking in papillary thyroid carcinoma \cite{33}. Lamin A/C overexpression has been implicated in human prostate cancer. It is believed to effect cell motility and growth via the PI3K/AKT/PTEN pathway \cite{10}. Defects in nuclear lobulations are well documented in human prostate cancer cell lines, which are described as lamin B deficient micro-domains (LMDM). Increased LMDMs correlate with more aggressive neoplastic behavior \cite{11}. Similarly, overexpression of lamin A/C is also seen in the context of colonic cancer \cite{34}. In this context, lamin A/C are thought to enable cell motility, thus contributing to increased aggressiveness of the disease \cite{35}. This may also be the case in ovarian cancer where increased levels of lamin A/C are seen \cite{36}.

On the other hand, deficient lamin A/C expression has been found in nodal diffuse large B-cell lymphoma, in gastric carcinoma, small cell lung carcinoma, basal cell carcinoma and in ovarian carcinoma \cite{35, 37-40}. In some of these cases, the deficiency of lamin A/C was thought to contribute to the observed chromosomal instability \cite{41-43}.
Recently, Wong et al. have suggested circulating LMNB1 mRNA as a biomarker for early detection of hepatocellular carcinoma in cirrhotic patients, with a 76% sensitivity and 82% specificity [44]. Others have seen this too [45]. Further lamin B1 has been seen to be affected in prostate [39, 46], cervical and uterine cancers [39].

To the best of our knowledge, we are the first group to present clinical data regarding the role of lamin A/C in human breast cancer. Our study is based on robust real-time PCR methodology, which we have employed in cohort with a median follow-up of ten years. The association of low expression of lamin A/C with advanced disease may suggest a significant role for chromosomal stability, lack of control on differentiation and cell ageing in human breast cancer. This requires further investigation with immunohistochemistry and mechanistic studies in cell lines to be better understand, especially in view of the conflicting role of mTOR in human breast cancer [47, 48].

In addition, we believe we are the first group report a role for lamin B1 and LBR in human breast cancer. We hope our findings would help guide further research in the role of nuclear envelope proteins in human breast cancer, which may open further avenues of enquiry into the mechanism underlying breast carcinogenesis and new therapeutics.

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