



# Prognostic Value of IMP3 and Its Role as an Epithelial-Mesenchymal Transition Promoter in Breast Carcinoma

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## Abstract

Breast carcinoma is now the most common cancer in the world. In view of its high mortality, there is a need to identify new prognostic biomarkers. Both IMP3 and SLUG have been implicated in cancer metastasis. This was a retrospective study conducted on 60 breast carcinoma cases using tissue microarrays. Demographic and clinicopathological details were recorded. Immunohistochemistry for IMP3 and SLUG was performed and evaluated in terms of percentage-cell-positivity and intensity of staining. A proforma was used to store data and was analyzed using SPSS v20. IMP3 positivity was found in 87% breast carcinoma cases and was significantly associated with tumor size ( $p = 0.03$ ) and TNM stage ( $p = 0.024$ ). IMP3 staining intensity showed significant association with histological grade ( $p = 0.009$ ), TNM stage ( $p = 0.036$ ), and molecular subtype ( $p = 0.03$ ). SLUG immunopositivity was seen in 90% breast carcinoma cases and was significantly associated with TNM stage ( $p = 0.006$ ). SLUG staining intensity was significantly associated with age ( $p = 0.025$ ) and TNM stage ( $p = 0.004$ ). IMP3 and SLUG immunopositivity and their staining intensities were significantly associated ( $p < 0.001$ ,  $p < 0.001$ ). IMP3 and SLUG percentage cell positivities were also significantly correlated ( $p < 0.001$ ). IMP3 and SLUG are, thus, poor prognostic markers with a role in tumor invasiveness and aggressiveness via epithelial-mesenchymal transition. Hence, IMP3 and SLUG-based targeted therapies may be useful in the treatment of breast carcinoma.

**Keywords** Breast carcinoma · IMP3 · SLUG · EMT · Prognostic markers · Immunohistochemistry

## Introduction

Breast carcinoma is the most common cancer occurring in women worldwide [1]. It is responsible for 24.2% of all female cancers globally and is the fifth most common cause of cancer-related deaths in females [2]. In view of the increasing prevalence, high mortality associated with breast cancer, and emergence of resistance to traditional chemotherapeutic drugs and hormonal therapy [3], there is a need

to identify new prognostic and predictive biomarkers that can be used as potential therapeutic targets in the future.

Insulin-like growth factor 2 (IGF2) messenger RNA (mRNA)-binding protein-3 (IMP3) is a highly conserved oncofetal RNA-binding protein (RBP) that regulates the steps of RNA processing. It has an essential role in RNA trafficking, cell growth, and cell migration during embryogenesis, but it is either completely absent or is expressed at very low levels after birth. On malignant transformation, IMP3 can re-express and resume its physiological functions within the malignant cells. Thus, it seems to play a role in tumor progression and maintenance of the hierarchies of tumor cells [4]. High IMP3 expression has been reported to be a predictor of metastasis and poor survival in various solid tumors including renal cell carcinoma, lung cancer, oral cancer, urothelial carcinoma, and hepatocellular carcinoma [5]. IMP3 is also known to induce epithelial-mesenchymal transition (EMT) by binding to SLUG mRNA [6]. EMT plays an important role in tumor invasion and metastasis. Transcription factors such as SNAIL, SLUG, and Twist,

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when aberrantly expressed, can induce EMT by repressing the transcription of E-cadherin [7, 8].

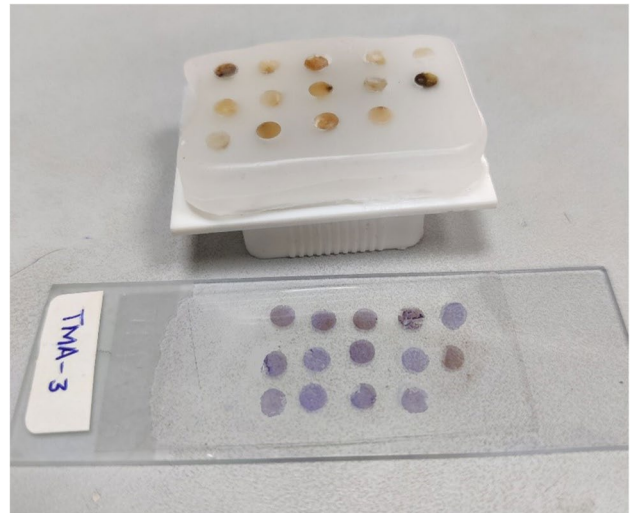
SLUG is a product of SNAI2 (snail family transcriptional repressor 2) and a member of the SNAIL-related family of zinc finger transcriptional repressors. It mediates sequence-specific interactions with DNA and is critical for the normal development of neural crest-derived cells. Aberrant induction of SLUG has been documented in cancer cells. It has been implicated in promoting tumor invasiveness by induction of the EMT. The role of SLUG in the progression of cell migration and tumor metastasis by promoting angiogenesis at the transcription level has also been reported [3]. SLUG has been reported to be an important indicator of tumor invasion and may be considered both a tumor marker as well as a therapeutic target for various malignancies [9].

Hence, we planned to assess the immunoeexpression of IMP3 and SLUG in breast carcinoma.

## Materials and Methods

This study was conducted in the Department of Pathology at tertiary healthcare center and teaching hospital in Delhi, between September 2019 and March 2021 after obtaining institutional ethical clearance (IEC-HR/2019/41/126). Diagnosed breast carcinoma cases in which the representative tumor tissue was available in the mastectomy specimens were included in the study, while cases showing non-availability of representative tumor tissue and having history of neoadjuvant chemotherapy or chemoradiation were excluded. Of 72 breast carcinoma cases diagnosed during the study period, 60 met our inclusion criteria. Clinicopathological and demographic details were noted from histopathology requisition forms. The routine hematoxylin and eosin-stained sections were reviewed to study the histological features of the tumor. Tumor grading was done using the Nottingham modification of the Bloom-Richardson system (Elston and Ellis criteria), and AJCC staging was used for tumor staging. For interpretation of ER, PR, and Her-2/neu status, routine reporting immunohistochemistry slides were re-evaluated on the basis of ASCO/CAP guidelines [10, 11].

Tissue blocks were prepared from the representative tumor tissue in all 60 cases. Tissue microarrays (TMA) were constructed using the Kononen et al. method. Each TMA had 12 tumor tissue cores and 2 control cores (pancreas for IMP3 and testis for SLUG) (Fig. 1). Immunohistochemistry for IMP-3 (rabbit polyclonal IgG anti-IMP3 protein antibody, ProteinTech Group, Rosemont, IL, USA) and SLUG (primary murine monoclonal IgG1 $\kappa$  anti-SLUG protein



**Fig. 1** A fully constructed TMA block and final immunostained section taken from the manual TMA constructed

antibody, SC-166476, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was done on sections prepared from the TMA blocks on lysinated slides. The cytoplasmic immunoeexpression of IMP3 and SLUG was considered positive, and immunopositivity was evaluated in terms of percentage of positively stained cells and staining intensity, which was expressed as mild, moderate, and intense.

Data was recorded in MS Excel sheet, and SPSS v20 was used for statistical analysis. Wilcoxon-Mann-Whitney *U*-test and Fisher's exact test were used to study the association between clinicopathological parameters and IMP3 and SLUG immunopositivity. Fisher's exact test was also used to study the association between immunoeexpression and staining intensities of IMP3 and SLUG. Spearman's correlation coefficient ( $\rho$ ) was used to assess the correlation between IMP3 and SLUG percentage cell positivity.

## Results

The demographic and clinicopathological details of the patients are summarized in Table 1.

### IMP3 Immunoeexpression

Out of 60, IMP3 immunopositivity was found in 52 (86.7%) breast carcinoma cases. Of these, 13 (25%) showed mild intensity of IMP3 positivity, 35 (67.3%) showed moderate, and 4 (7.7%) showed intense immunostaining for IMP3

**Table 1** Demographic and clinicopathological parameters of breast carcinoma patients

| All parameters                    | Frequency (%) |
|-----------------------------------|---------------|
| <b>Age</b>                        |               |
| 21–29 years                       | 1 (1.7%)      |
| 30–39 years                       | 15 (25.0%)    |
| 40–49 years                       | 25 (41.7%)    |
| 50–59 years                       | 8 (13.3%)     |
| >60 years                         | 11 (18.3%)    |
| <b>Menstrual status</b>           |               |
| Pre-menopausal                    | 25 (41.7%)    |
| Post-menopausal                   | 35 (58.3%)    |
| <b>Laterality</b>                 |               |
| Right                             | 30 (50.0%)    |
| Left                              | 30 (50.0%)    |
| <b>Size</b>                       |               |
| ≤ 2 cm                            | 13 (21.7%)    |
| > 2–5 cm                          | 26 (43.3%)    |
| > 5 cm                            | 21 (35.0%)    |
| <b>Lymph node</b>                 |               |
| Absent                            | 24 (40.0%)    |
| pN1                               | 16 (26.7%)    |
| pN2                               | 12 (20.0%)    |
| pN3                               | 8 (13.3%)     |
| In situ component (present)       | 21 (35.0%)    |
| Lymphovascular invasion (present) | 18 (30.0%)    |
| Extranodal extension (present)    | 10 (16.7%)    |
| <b>Histological grade</b>         |               |
| I                                 | 21 (35.0%)    |
| II                                | 20 (33.3%)    |
| III                               | 19 (31.7%)    |
| <b>Primary tumor</b>              |               |
| pT1                               | 14 (23.3%)    |
| pT2                               | 22 (36.7%)    |
| pT3                               | 16 (26.7%)    |
| pT4                               | 8 (13.3%)     |
| <b>TNM stage</b>                  |               |
| I                                 | 9 (15.0%)     |
| II                                | 24 (40.0%)    |
| III                               | 27 (45.0%)    |
| ER status (positive)              | 31 (51.7%)    |
| PR status (positive)              | 31 (51.7%)    |
| Her/Neu 2 status (positive)       | 27 (45.0%)    |
| <b>Molecular subtype</b>          |               |
| Luminal                           | 31 (51.7%)    |
| Her-2/neu enriched                | 14 (23.3%)    |
| Triple negative                   | 15 (25.0%)    |

(Fig. 2). The mean percentage cell positivity for IMP3 in breast carcinoma cases was 80.25%. IMP3 immunopositivity revealed significant association with tumor size ( $p=0.03$ ) and TNM stage ( $p=0.02$ ) of breast carcinoma. Further, IMP3 staining intensity was significantly associated with histological grade ( $p=0.009$ ,  $\chi^2=14.28$ ), TNM staging ( $p=0.03$ ,  $\chi^2=13.59$ ), and molecular subtype ( $p=0.03$ ,  $\chi^2=13.67$ ). The IMP3 immunopositivity in breast carcinoma and its association with demographic and clinicopathological parameters is shown in Table 2.

### SLUG Immunopositivity

SLUG immunopositivity was found in 54/60 (90%) breast carcinoma cases. Of these, 6 (11.1%) cases showed mild intensity of SLUG positivity; 34 (63%) cases showed moderate intensity, and intense immunostaining for SLUG was found in 14 (25.9%) cases (Fig. 3). The mean percentage cell positivity for SLUG in breast carcinoma cases was 87.42%. SLUG immunopositivity was found to be significantly associated with the TNM stage of breast carcinoma ( $p=0.06$ ). Significant association of SLUG immunostaining intensity was also found with the age of the patients ( $p=0.025$ ,  $\chi^2=25.02$ ) and TNM stage of breast carcinoma ( $p=0.004$ ,  $\chi^2=17.415$ ). SLUG immunopositivity in breast carcinoma and its association with demographic and clinicopathological parameters is shown in Table 3.

### Association Between IMP3 and SLUG Immunopositivity

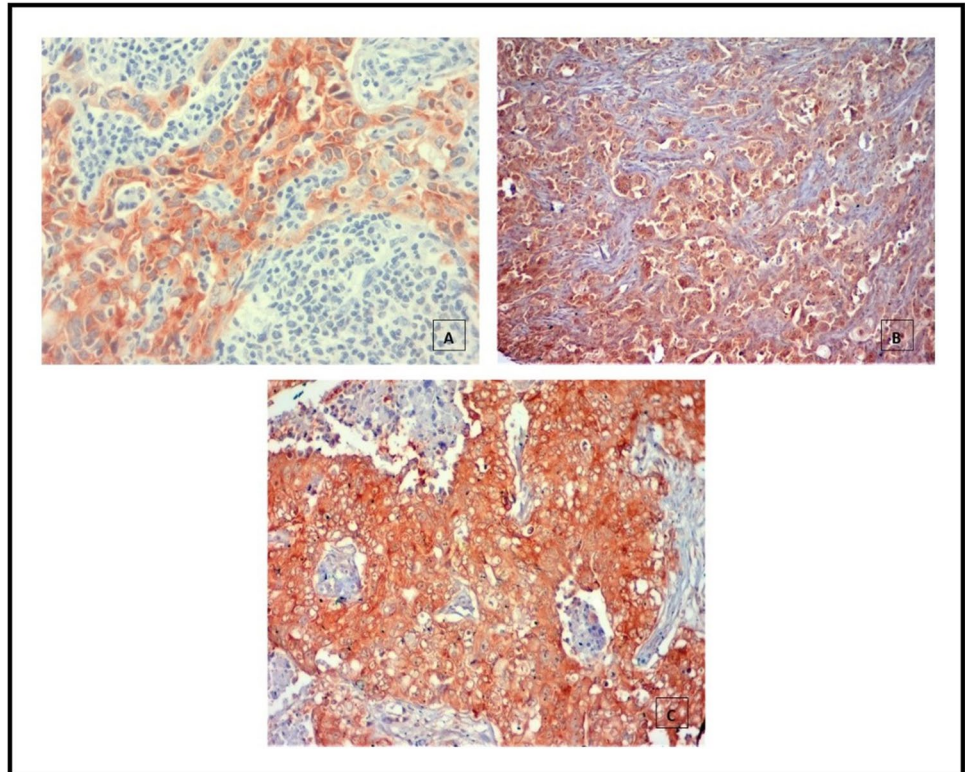
Immunopositivity of IMP3 was found to have significant association with SLUG expression in breast carcinoma ( $p<0.001$ ,  $\chi^2=43.33$ ), as shown in Table 4.

Moderate positive correlation was found between SLUG percentage cell positivity and IMP3 percentage cell positivity (Fig. 4) and was statistically significant ( $\rho=0.44$ ,  $p<0.001$ ).

### Discussion

Breast cancer, a heterogeneous disease, is now the most common cancer worldwide [12]. Many limitations have been identified in the currently available treatment modalities for breast carcinoma due to molecular evolution and tumor heterogeneity, changes in intrinsic subtypes during metastasis, and development of post-treatment complications [13, 14]. High IMP3 expression is a predictor of metastasis and poor survival

**Fig. 2** Photomicrograph shows **A** mild intensity of IMP3 cytoplasmic immunopositivity in grade 1 breast carcinoma, **B** moderate intensity of IMP3 immunopositivity in grade 2 breast carcinoma, and **C** intense cytoplasmic staining of IMP3 in grade 3 breast carcinoma (IHC: IMP3, 400×)



[5, 15]. The role of IMP3 as an EMT promoter by binding to SLUG mRNA has been documented in literature [6].

Majority of the studies in literature have reported IMP3 positivity in breast carcinoma ranging from 33 to 35.6% [16–18]. Contrary to this, in the present study, IMP3 immunopositivity was seen in 86.7% of the breast carcinoma cases. This could be explained by geographical and demographic differences.

We found that IMP3 positivity was significantly higher in tumors of greater size ( $p < 0.05$ ) and higher TNM stage ( $p < 0.05$ ). IMP3 staining intensity also showed significant association with tumor histological grade ( $p = 0.009$ ) and TNM stage ( $p = 0.036$ ). Sjekloča et al. also reported a significant association between IMP3 positivity and tumor size and TNM stage [18]. Similar to our study results, Walter et al. also found a significantly higher expression of IMP3 in higher grade breast tumors ( $p < 0.001$ ) [16]. In cancers other than breast cancer as well, a higher TNM stage is associated with IMP3 positivity [19, 20].

There are few studies in literature that have evaluated the IMP3 immunopositivity and staining intensity in different molecular subtypes of breast carcinoma. IMP3 expression in breast carcinoma has been mainly studied in triple negative breast cancer (TNBC). It has been reported that IMP3 promotes TNBC stem cell property [21, 22]. In the present study, the highest IMP3 positivity was seen in the luminal subtype followed by the TNBC subtype. However, this association was not statistically significant. IMP3 staining

intensity, however, showed significant association with molecular subtype ( $p < 0.05$ ) with the highest staining intensity seen in Her-2/neu-enriched subtype followed by TNBC. This could be explained by geographical and demographic differences in molecular subtyping of breast carcinoma and the smaller population of TNBC cases in our study.

No significant association was found between IMP3 positivity or staining intensity and other clinicopathological parameters like tumor laterality, menopausal status, in situ component, lymphovascular invasion, lymph node status, and ER/PR and HER-2/neu status. However, Walter et al. found significant association between IMP3 expression and ER, PR, and Her-2/neu-negative status, which could probably be explained by the entirely TNBC nature of their study population [16]. Sufficient information regarding the association of the rest of these clinicopathological characteristics with IMP3 is unavailable in current literature.

Previous studies on breast carcinoma have reported SLUG immunopositivity ranging from 34 to 100% [23–25]. In the present study, we found SLUG to be positive in 90% breast cancer cases, which is in line with the previous study results [24, 26].

SLUG positivity was significantly higher ( $p < 0.01$ ) in TNM stage III. SLUG staining intensity was also found to have significant association with TNM stage ( $p < 0.01$ ). This is in concordance with the findings of other studies [24, 26] but contrary to study results of Gu et al. as they found greater number of stage I and II cases to be SLUG positive [23].



**Table 2** Association between IMP3 immunoeexpression and demographic and clinicopathological characteristics in breast carcinoma cases (n=60)

| Parameters                        | IMP3 immunoeexpression |                  | p value            |
|-----------------------------------|------------------------|------------------|--------------------|
|                                   | Positive (n = 52)      | Negative (n = 8) |                    |
| Age (years)                       | 47.08 ± 10.87          | 47.25 ± 14.74    | 0.991 <sup>1</sup> |
| Age                               |                        |                  | 0.164 <sup>2</sup> |
| 21–29 years                       | 0 (0.0%)               | 1 (12.5%)        |                    |
| 30–39 years                       | 14 (26.9%)             | 1 (12.5%)        |                    |
| 40–49 years                       | 21 (40.4%)             | 4 (50.0%)        |                    |
| 50–59 years                       | 8 (15.4%)              | 0 (0.0%)         |                    |
| >60 years                         | 9 (17.3%)              | 2 (25.0%)        |                    |
| Menstrual status                  |                        |                  | 1.000 <sup>2</sup> |
| Pre-menopausal                    | 22 (42.3%)             | 3 (37.5%)        |                    |
| Post-menopausal                   | 30 (57.7%)             | 5 (62.5%)        |                    |
| Laterality                        |                        |                  | 0.706 <sup>2</sup> |
| Right                             | 27 (51.9%)             | 3 (37.5%)        |                    |
| Left                              | 25 (48.1%)             | 5 (62.5%)        |                    |
| Size***                           |                        |                  | 0.030 <sup>2</sup> |
| ≤ 2 cm                            | 13 (25.0%)             | 0 (0.0%)         |                    |
| > 2–5 cm                          | 19 (36.5%)             | 7 (87.5%)        |                    |
| > 5 cm                            | 20 (38.5%)             | 1 (12.5%)        |                    |
| Lymph node                        |                        |                  | 0.645 <sup>2</sup> |
| Absent                            | 19 (36.5%)             | 5 (62.5%)        |                    |
| pN1                               | 14 (26.9%)             | 2 (25.0%)        |                    |
| pN2                               | 11 (21.2%)             | 1 (12.5%)        |                    |
| pN3                               | 8 (15.4%)              | 0 (0.0%)         |                    |
| In situ component (present)       | 17 (32.7%)             | 4 (50.0%)        | 0.433 <sup>2</sup> |
| Lymphovascular invasion (present) | 16 (30.8%)             | 2 (25.0%)        | 1.000 <sup>2</sup> |
| Extranodal extension (present)    | 10 (19.2%)             | 0 (0.0%)         | 0.330 <sup>2</sup> |
| Histological grade                |                        |                  | 0.636 <sup>2</sup> |
| I                                 | 19 (36.5%)             | 2 (25.0%)        |                    |
| II                                | 16 (30.8%)             | 4 (50.0%)        |                    |
| III                               | 17 (32.7%)             | 2 (25.0%)        |                    |
| TNM stage***                      |                        |                  | 0.024 <sup>2</sup> |
| I                                 | 9 (17.3%)              | 0 (0.0%)         |                    |
| II                                | 17 (32.7%)             | 7 (87.5%)        |                    |
| III                               | 26 (50.0%)             | 1 (12.5%)        |                    |
| ER status (positive)              | 25 (48.1%)             | 6 (75.0%)        | 0.257 <sup>2</sup> |
| PR status (positive)              | 25 (48.1%)             | 6 (75.0%)        | 0.257 <sup>2</sup> |
| Her/Neu 2 status (positive)       | 22 (42.3%)             | 5 (62.5%)        | 0.448 <sup>2</sup> |
| Molecular subtype                 |                        |                  | 0.409 <sup>2</sup> |
| Luminal                           | 25 (48.1%)             | 6 (75.0%)        |                    |
| Her-2/neu enriched                | 13 (25.0%)             | 1 (12.5%)        |                    |
| Triple negative                   | 14 (26.9%)             | 1 (12.5%)        |                    |

\*\*\*Significant at p<0.05

<sup>1</sup>Wilcoxon-Mann-Whitney U-Test

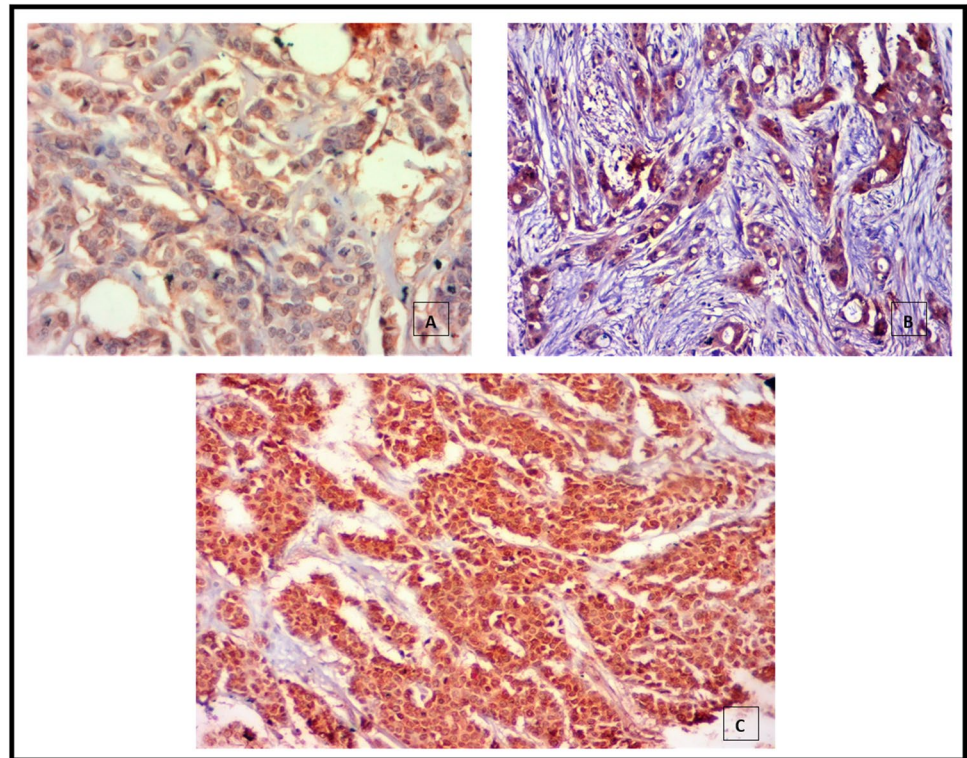
<sup>2</sup>Fisher’s exact test

SLUG positivity was found to be non-significantly higher in patients with lymph node involvement. However, Fouad et al. and Côme et al. reported a significant correlation between lymph node status and SLUG positivity [24, 27]. In contrast, Gu et al. found that higher SLUG expression is

found in lymph node-negative cases [23]. This discordance in findings can be explained by the variation in population demographics.

We found SLUG positivity to be highest in luminal subtype, followed by TNBC subtype and least in Her-2/

**Fig. 3** Photomicrograph shows (A) mild intensity of SLUG cytoplasmic immunopositivity in grade 1 breast carcinoma, (B) moderate intensity of SLUG immunopositivity in grade 2 breast carcinoma, and C diffuse intense cytoplasmic immunostaining for SLUG in grade 3 breast carcinoma (IHC: SLUG, 400×)



neu-enriched cases. Similar findings were reported by Fouad et al., while Gu et al. showed a statistically significant strong correlation between TNBC subtype and SLUG positivity [23, 24]. Thus, more studies with larger sample size are required to establish the relationship between SLUG and molecular subtypes of breast cancer. Further, the small population of cancers of the TNBC subtype in our study could also account for this discrepancy.

No significant association was found between SLUG positivity or staining intensity and other factors like menopausal status, tumor laterality, size, grade, in situ component, lymphovascular invasion, ER/PR and HER-2 status, and molecular subtype. Fouad et al., however, found that SLUG expression was higher in ER- and PR-negative cases [24]. This difference could be possibly due to differences in population characteristics. Sufficient information regarding the association of the rest of these clinicopathological characteristics with SLUG is not available in current literature.

We found a highly significant association ( $p < 0.001$ ) between IMP3 and SLUG immunopositivity with 86.67% cases showing positivity for both. All IMP3-positive cases were found to be SLUG positive. IMP3 induces EMT by binding SLUG mRNA, which may explain the association found between the two proteins. However, out of 54 SLUG-positive cases, 2 were IMP3 negative, thus implicating a minor pathway of SLUG expression

that is unrelated to IMP3. SLUG is also known to be regulated by other proteins such as TGF- $\beta$  and GSK-3 $\beta$ , which may further explain this [28]. IMP3 and SLUG staining intensities also showed highly significant correlation ( $p < 0.001$ ). These findings are in accordance with studies conducted by Su et al. and Samanta et al. where they studied the relationship between IMP3 and SLUG in breast carcinoma via mRNA-based studies [29, 30]. Samanta et al. discovered that IMP3 binds avidly to SLUG mRNA, hence, regulating its expression by binding to the 5' UTR. SLUG, in turn, targets SOX2, which is a stem cell factor. Thus, establishing a mechanism of breast tumor initiation involving IMP3 and providing a basis for its association with aggressiveness and poor outcome of breast carcinoma [30]. Su et al. found that IMP3 expression directly correlated with both SLUG ( $p = 0.004$ ) and vimentin ( $p < 0.001$ ) suggesting that SLUG acts as a functional target of IMP3. In the present study, our findings are also in accordance with these.

### Limitations

In our study, however, there was a lack of follow-up for disease recurrence and progression after modified radical mastectomy due to the outbreak of the COVID-19 pandemic. Hence, disease-free survival and overall survival could not be calculated. Further, our study was retrospective and also

**Table 3** Association between SLUG immunostaining and demographic and clinicopathological characteristics in breast carcinoma (n=60)

|                                   | SLUG inference    |                  | p value            |
|-----------------------------------|-------------------|------------------|--------------------|
|                                   | Positive (n = 54) | Negative (n = 6) |                    |
| Age (years)                       | 47.06 ± 10.67     | 47.50 ± 17.41    | 0.882 <sup>1</sup> |
| Age                               |                   |                  | 0.124 <sup>2</sup> |
| 21–29 years                       | 0 (0.0%)          | 1 (16.7%)        |                    |
| 30–39 years                       | 14 (25.9%)        | 1 (16.7%)        |                    |
| 40–49 years                       | 23 (42.6%)        | 2 (33.3%)        |                    |
| 50–59 years                       | 8 (14.8%)         | 0 (0.0%)         |                    |
| >60 years                         | 9 (16.7%)         | 2 (33.3%)        |                    |
| Menstrual status                  |                   |                  | 0.686 <sup>2</sup> |
| Pre-menopausal                    | 22 (40.7%)        | 3 (50.0%)        |                    |
| Post-menopausal                   | 32 (59.3%)        | 3 (50.0%)        |                    |
| Laterality                        |                   |                  | 0.671 <sup>2</sup> |
| Right                             | 28 (51.9%)        | 2 (33.3%)        |                    |
| Left                              | 26 (48.1%)        | 4 (66.7%)        |                    |
| Size                              |                   |                  | 0.138 <sup>2</sup> |
| ≤ 2 cm                            | 13 (24.1%)        | 0 (0.0%)         |                    |
| > 2–5 cm                          | 21 (38.9%)        | 5 (83.3%)        |                    |
| > 5 cm                            | 20 (37.0%)        | 1 (16.7%)        |                    |
| Lymph node                        |                   |                  | 0.454 <sup>2</sup> |
| Absent                            | 20 (37.0%)        | 4 (66.7%)        |                    |
| pN1                               | 14 (25.9%)        | 2 (33.3%)        |                    |
| pN2                               | 12 (22.2%)        | 0 (0.0%)         |                    |
| pN3                               | 8 (14.8%)         | 0 (0.0%)         |                    |
| In situ component (present)       | 19 (35.2%)        | 2 (33.3%)        | 1.000 <sup>2</sup> |
| Lymphovascular invasion (present) | 16 (29.6%)        | 2 (33.3%)        | 1.000 <sup>2</sup> |
| Extranodal extension (present)    | 10 (18.5%)        | 0 (0.0%)         | 0.577 <sup>2</sup> |
| Histological grade                |                   |                  | 0.300 <sup>2</sup> |
| I                                 | 20 (37.0%)        | 1 (16.7%)        |                    |
| II                                | 16 (29.6%)        | 4 (66.7%)        |                    |
| III                               | 18 (33.3%)        | 1 (16.7%)        |                    |
| Primary tumor                     |                   |                  | 0.608 <sup>2</sup> |
| pT1                               | 13 (24.1%)        | 1 (16.7%)        |                    |
| pT2                               | 18 (33.3%)        | 4 (66.7%)        |                    |
| pT3                               | 15 (27.8%)        | 1 (16.7%)        |                    |
| pT4                               | 8 (14.8%)         | 0 (0.0%)         |                    |
| TNM stage***                      |                   |                  | 0.006 <sup>2</sup> |
| I                                 | 9 (16.7%)         | 0 (0.0%)         |                    |
| II                                | 18 (33.3%)        | 6 (100.0%)       |                    |
| III                               | 27 (50.0%)        | 0 (0.0%)         |                    |
| ER status (positive)              | 27 (50.0%)        | 4 (66.7%)        | 0.672 <sup>2</sup> |
| PR status (positive)              | 27 (50.0%)        | 4 (66.7%)        | 0.672 <sup>2</sup> |
| Her/Neu 2 status (positive)       | 24 (44.4%)        | 3 (50.0%)        | 1.000 <sup>2</sup> |
| Molecular subtype                 |                   |                  | 1.000 <sup>2</sup> |
| Luminal                           | 27 (50.0%)        | 4 (66.7%)        |                    |
| Her-2/neu enriched                | 13 (24.1%)        | 1 (16.7%)        |                    |
| Triple negative                   | 14 (25.9%)        | 1 (16.7%)        |                    |

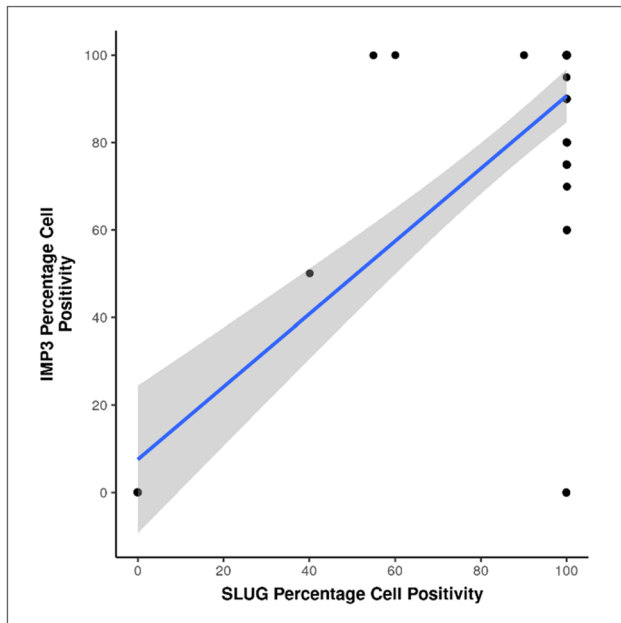
\*\*\*Significant at  $p < 0.05$

<sup>1</sup>Wilcoxon-Mann-Whitney *U*-test

<sup>2</sup>Fisher’s exact test

**Table 4** Association between IMP3 and SLUG staining intensities (*n* = 60)

|          | SLUG expression |             | IMP3 expression |            |             | Fisher's exact test |                |
|----------|-----------------|-------------|-----------------|------------|-------------|---------------------|----------------|
|          | Negative        | Mild        | Moderate        | Intense    | Total       | $\chi^2$            | <i>p</i> value |
| Negative | 6 (75.0%)       | 0 (0.0%)    | 0 (0.0%)        | 0 (0.0%)   | 6 (10.0%)   | 51.132              | <0.001         |
| Mild     | 1 (12.5%)       | 3 (23.1%)   | 2 (5.7%)        | 0 (0.0%)   | 6 (10.0%)   |                     |                |
| Moderate | 1 (12.5%)       | 9 (69.2%)   | 22 (62.9%)      | 2 (50.0%)  | 34 (56.7%)  |                     |                |
| Intense  | 0 (0.0%)        | 1 (7.7%)    | 11 (31.4%)      | 2 (50.0%)  | 14 (23.3%)  |                     |                |
| Total    | 8 (100.0%)      | 13 (100.0%) | 35 (100.0%)     | 4 (100.0%) | 60 (100.0%) |                     |                |



**Fig. 4** Scatterplot depicting correlation between SLUG percentage cell positivity and IMP3 percentage cell positivity. Individual points represent individual cases. The blue trendline represents the general trend of correlation between the two variables. The shaded gray area represents the 95% confidence interval of this trendline

had a small cohort due to constraints of time, resources, and the pandemic.

**Conclusion**

Significant association of immunoexpression of IMP3 and SLUG with TNM stage proves them to be poor prognostic markers. Also, significant positive correlation between immunoexpression of IMP3 and SLUG highlights their proposed role in tumor invasiveness and aggressiveness via EMT in breast carcinomas. Hence, IMP3 and SLUG-based targeted therapies might prove to be useful in the treatment of breast carcinoma

**Author contributions**

| Contributors                       | 1 | 2 | 3 | 4 |
|------------------------------------|---|---|---|---|
| Concepts                           | * | * | * | * |
| Design                             | * | * | * | * |
| Definition of intellectual content | * | * | * | * |
| Literature search                  | * |   |   |   |
| Experimental studies               | * |   |   |   |
| Data acquisition                   | * | * |   |   |
| Data analysis                      | * | * |   |   |
| Manuscript preparation             | * |   |   |   |
| Manuscript editing                 | * | * | * | * |
| Manuscript review                  | * | * | * | * |

**Declarations**

**Conflict of Interest** The authors declare no competing interests.

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