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INTERPLAY OF SNAIL/SLUG, AND NEUROPILIN-2 WITH HISTOPATHOLOGICAL AND HORMONAL FACTORS IN PREDICTING LYMPH NODE METASTASIS IN INVASIVE DUCTAL CARCINOMA - A RETROSPECTIVE STUDY

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ABSTRACT

Background: Invasive ductal carcinoma (IDC) is the most prevalent histological subtype of breast cancer and is characterized by marked biological heterogeneity. Epithelial-mesenchymal transition (EMT)-related proteins, including SNAIL/SLUG, and Neuropilin-2 (NRP2), have been implicated in tumor invasion, progression, and metastatic dissemination.

Aim: To evaluate the immunohistochemical expression of SNAIL/SLUG, and NRP2 in invasive ductal carcinoma and to investigate their association with lymph node metastasis, clinicopathological characteristics, and hormonal receptor status.

Materials and Methods: A retrosepective observational study was conducted on 80 patients with histopathologically confirmed invasive ductal carcinoma who underwent mastectomy between January 2022 and December 2025. Immunohistochemical staining for SNAIL/SLUG and NRP2 was performed, and expression patterns were correlated with tumor grade, lymph node status, estrogen receptor (ER), progesterone receptor (PR), HER2/neu status, and Ki-67 proliferative index.

Results: Among 80 invasive ductal carcinoma cases, high NRP2 and SNAIL/SLUG expression was observed in 27.5% and 40.0% of tumors, respectively. Significant co-expression of both markers was demonstrated (OR=7.00, $p<0.001$), with a positive correlation between their numerical expression scores ($\rho=0.372$, $p<0.001$). High SNAIL/SLUG expression was significantly associated with ER negativity (57.1% vs 26.7%, $p=0.011$). NRP2 expression was higher in node-positive tumors, although the association was not statistically significant. Both markers showed higher expression in triple-negative tumors.

Conclusion: NRP2 and SNAIL/SLUG demonstrate significant biological interplay in invasive ductal carcinoma and may contribute to aggressive tumor behavior. Their association with ER negativity and increased expression in triple-negative tumors suggests potential value as biomarkers of aggressive disease and lymphatic metastatic potential. Further larger-scale studies are warranted to establish their independent prognostic and predictive significance.

KEYWORDS: Invasive ductal carcinoma; Breast cancer; Snail; Slug; Neuropilin-2; Epithelial–mesenchymal transition; Lymph node metastasis.

INTRODUCTION

Breast cancer remains one of the most frequently diagnosed malignancies worldwide and represents a major cause of cancer-related morbidity and mortality among women. Despite improvements in screening, pathological assessment, systemic therapy, and targeted treatment, lymph node metastasis remains one of the most important determinants of disease stage, recurrence risk, and therapeutic decision-making. Regional lymphatic spread is particularly relevant in invasive ductal carcinoma (IDC), where the biological ability of tumor cells to invade surrounding tissue and enter lymphatic channels may differ considerably between tumors with otherwise similar conventional histopathological characteristics. Recent research has therefore focused on identifying molecular markers that can complement established clinicopathological parameters in predicting metastatic behavior. [1], [2]

Tumor progression and metastasis involve a complex interaction between malignant cells, the extracellular matrix, lymphatic vasculature, and the tumor microenvironment. Epithelial–mesenchymal transition (EMT) is an important biological process implicated in acquisition of migratory and invasive properties. During EMT, epithelial tumor cells undergo changes in cell adhesion and phenotype that facilitate local invasion and dissemination. The transcription factors SNAIL and SLUG are among the principal regulators of this process and can suppress epithelial characteristics while promoting mesenchymal behavior. Contemporary reviews have emphasized that SNAIL/SLUG-related signaling converges with several pathways involved in breast cancer invasion, metastatic spread, stemness, and treatment resistance. [3], [4]

The clinical relevance of SLUG is supported by accumulating evidence. A 2022 systematic review and meta-analysis found that increased SLUG expression was associated with poorer overall and disease-free survival, higher TNM stage, greater likelihood of axillary lymph node metastasis, and estrogen-receptor deficiency. These observations suggest that SLUG may provide information beyond conventional histopathological grading and may identify tumors with a more aggressive phenotype. [5] SNAIL and SLUG expression may also vary according to molecular subtype, with increased EMT-marker expression reported particularly in biologically aggressive breast cancers.

Neuropilin-2 (NRP2) has emerged as another potentially important mediator of breast cancer progression. NRP2 is a transmembrane co-receptor involved in signaling pathways regulating tumor-cell migration, angiogenesis, lymphangiogenesis, and metastatic dissemination. Experimental evidence has demonstrated that NRP2-related signaling can contribute to aggressive behavior in triple-negative breast cancer, while therapeutic inhibition of VEGF–NRP2 interaction has been shown to reduce metastatic potential in preclinical models. [6] These findings provide a biological rationale for investigating NRP2 alongside EMT-associated transcription factors.

The present Retrospective observational study has undertaken to evalvate the interplay of SNAIL,SLUG, and NRP2 with histopathological and hormonal factors in predicting lymph node metastasis in IDC. By examining these molecular markers together with tumor size, histological grade, TNM stage, lymph node status, ER, PR, HER2, and Ki-67, the study aims to clarify whether their combined expression can identify tumors with increased metastatic potential and provide clinically meaningful prognostic information.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology, RL Jalappa Hospital and Research Centre, Tamaka, Kolar, Karnataka, India, a tertiary care teaching hospital affiliated with Sri Devaraj Urs Academy of Higher Education and Research. The study was approved by the Institutional Ethics Committee (IEC No: SDUAHER/R&D/CEC/SDUMC-PG/404/NF/- 2025-26). As this was a retrospective analysis of archival pathological specimens, the requirement for informed consent was waived by the ethics committee.

Study population: Patients diagnosed with invasive ductal carcinoma (invasive carcinoma of no special type, NST) who underwent mastectomy with axillary lymph node dissection and had complete histopathological and hormonal receptor data available. The FISH was not done for equivocal cases.

Sample size: Using Difference of Proportions method for Hypothesis Testing.

We derive the expected proportions from the results reported in the study by Sherrin Jacob et al,[9] it showed a significant association between Snail+Slug expression and Nodal Stage (p=0.043).5

Group 1 (Node Negative / N0):

Total N0 patients: 25

High Snail+Slug expression: 8

Proportion (P1): 8/25 = 0.32

Group 2 (Node Positive / N1-N3):

Total N+ patients (N1+N2+N3): 13 + 12 + 8 = 33

High Snail+Slug expression: 6 + 9 + 6 = 21

Proportion (P2): 21/33 = 0.636

Statistical Assumptions

Confidence Level (1-α): 95% (Z = 1.96)

Power (1-β): 80% (Z = 0.84)

Ratio: 1:1 ratio between Node Negative and Node Positive groups (idealized for calculation).

Calculation

n=(〖(Z_(α⁄2)+Z_β)〗 ^2×[P_1 (1-P_1)+P_2 (1-P_2)])/((〖P_1-P_2)〗 ^2)

Substituting in the numbers:

Difference (P2 - P1): 0.636 - 0.320 = 0.316

Pooled Variance:

P1(1-P1) = 0.32 x 0.68 = 0.2176

P2(1-P2) = 0.636 x 0.364 = 0.2315

Sum = 0.4491

Z-score sum squared: (1.96 + 0.84)2 = 7.84

n=(〖(7.84)〗 ^2×0.4491)/((〖0.316)〗 ^2)≈36

36 patients per group (Node Negative vs. Node Positive).

Calculated Minimum: 72 patients (36 Node Negative + 36 Node Positive).

Attrition Buffer (10%): Adding ~8 patients to account for potential tissue loss

Total Sample Size: 80 Patients

Study setting/location: Department of Pathology, Sri Devaraj Urs Medical College (SDUMC), Kolar, using archived mastectomy specimens obtained from R. L. Jalappa Hospital between January 2022 and December 2025.

Inclusion criteria:

- Patients with histologically confirmed invasive carcinoma of no special type (NST).
- Mastectomy specimens with axillary lymph nodes available for evaluation.

- Complete immunohistochemical data for ER, PR, HER2/neu, and Ki-67.
- Adequate formalin-fixed, paraffin-embedded tissue blocks available for immunohistochemical analysis of Snail, Slug, and NRP2.

Exclusion criteria:
- Lumpectomy specimens without lymph node dissection.
- Histological subtypes other than invasive carcinoma, NST.
- Tru-cut biopsy specimens.
- Mastectomy specimens without lymph nodes.
- Patients who received neoadjuvant chemotherapy before surgery.

Study Procedure:
Specimen processing and histopathological examination: All modified radical mastectomy specimens were received fresh in the Department of Pathology and fixed in 10% neutral buffered formalin for 24-48 hours. Gross examination was performed in accordance with the College of American Pathologists (CAP) guidelines. Representative tissue sections, including the primary tumor, surgical margins, nipple areolar complex and all identified axillary lymph nodes, were sampled. The tissues were processed using standard histopathological techniques, embedded in paraffin wax, sectioned at 4-5 µm thickness, and stained with Haematoxylin and Eosin (H&E) for microscopic evaluation.
Axillary lymph nodes were meticulously identified from the axillary fat, individually dissected, measured, and processed separately. Histopathological examination was performed to assess nodal metastatic involvement and other relevant morphological features.Tumor staging was performed according to the TNM classification of breast carcinoma based on the American Joint Committee on Cancer (AJCC), 8th edition. Pathological tumor (pT), regional lymph node (pN), and overall pathological stage group were assigned based on the histopathological findings and available clinical information

Immunohistochemistry
The immunohistochemistry will be done using Estrogen receptor ID5 Clone, IgG1 Kappa. Progesterone receptor, clone EP2,and HER2/neu (ErB-2 oncoprotein), clone EP3. The scoring of ER and PR evaluation will be done based on Allred Score and HER/neu scoring based on ASCO Guidelines the slides will be retrieved for evaluation.HER2 was categorized as negative (0/1+), equivocal (2+), or positive (3+) according to ASCO/CAP criteria. Fluorescence in situ hybridization (FISH) or other confirmatory in situ hybridization testing was not performed for HER2-equivocal (2+) cases. Therefore, HER2-equivocal cases were excluded from molecular subtype classification[7,8]
Appropriate sections will be chosen for Immunohistochemistry of SLUG/SNAIL and NRP2.
The peroxidase-antiperoxidase technique will be employed as per the manufacturer's protocol.
Antibody Clone Species Producer Control Stain
Slug-snail Polyclonal Rabbit, Abcam (ab180714),Pancreas as positive control , Nuclear staining in tumor cells.
Neuropilin-2 Polyclonal Rabbit Elabscience (Catalog No: E-AB-16638) Cervical carcinoma / Thyroid carcinoma Cytoplasmic ± membranous staining in tumor cells

SNAIL/SLUG stain interpretation: SNAIL/SLUG expression was assessed using score adapted from Jacob S et al. [9]
Tumor cells were evaluated according to staining intensity as
negative (0),
weak (1+),
moderate (2+),
strong (3+).
The H-score was calculated as: (1 × percentage of weakly stained cells) + (2 × percentage of moderately stained cells) + (3 × percentage of strongly stained cells)Score = (1 x percentage weakly stained) + (2 x percentage moderately stained) + (3x percentage strongly stained)
H-Score Interpretation :Low when the H-score was ≤165
High when the H-score was >165

NRP2 Stain interpretation:NRP2 expression was assessed using an intensity–percentage score adapted from Dong et al .[10]
Intensity of Staining
0 Negative
1 Weak
2 Moderate
3 Strong
Parameter Score Criteria
Percentage of positive Tumor Cells
1= 0-10%
2= 11–50%
3 =51–80%
4= 81-100%
Final score : Intensity score × Percentage score

Expression category : Final score ≤3=Low
Final score >3= High

Statistical Analysis: Statistical analysis was performed using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA,Data from 80 invasive breast carcinoma cases with complete NRP2 and SNAIL/SLUG immunohistochemical scores were analyzed. Categorical variables are presented as frequencies and percentages. Continuous variables are summarized using mean and standard deviation or median and interquartile range, as appropriate.
NRP2 expression was categorized as low when the final immunohistochemical score was ≤3 and high when the score was >3. SNAIL/SLUG expression was categorized as low when the H-score was ≤165 and high when the H-score was >165, in accordance with the prespecified study protocol.
Associations between categorical marker expression and clinicopathological or hormonal variables were assessed using the Chi-square test. Fisher's exact test was used when expected cell frequencies were small. The strength of co-expression between high NRP2 and high SNAIL/SLUG was additionally expressed as an odds ratio.
Because the immunohistochemical expression scores are ordinal/non-normally distributed, the relationship between numerical NRP2 and SNAIL/SLUG scores was assessed using Spearman's rank correlation coefficient (ρ). Spearman correlation was also used for exploratory assessment of numerical marker scores with age and tumor size.
ER and PR were interpreted from the recorded Allred scores, with scores 0–2 considered negative and 3–8 positive. HER2 was categorized as negative (0/1+), equivocal (2+) or positive (3+). Molecular subtype analysis was restricted to tumors with unequivocal HER2 results because confirmatory ISH/FISH results were not available for HER2-equivocal cases.
All tests were two-tailed. A p value <0.05 was considered statistically significant. Borderline associations (approximately p=0.05–0.10) are described as trends and not interpreted as statistically significant.

RESULTS
The present study included 80 patients diagnosed with IDC-NST, who underwent mastectomy with axillary lymph node dissection.
The mean age of the patients was 52.2 ± 12.7 years, with 33 (41.2%) patients aged ≤50 years and 47 (58.8%) aged >50 years. The mean tumour size was 5.6 ± 2.8 cm. Histologically, Grade I tumours constituted the majority (44/80, 55%), followed by Grade II (31/80, 38.8%) and Grade III (5/80, 6.3%). According to TNM staging, 2 (2.5%) cases were classified as Stage I, 41 (51.2%) as Stage II, and 37 (46.2%) as Stage III. Lymph node metastasis was present in 40 (50.0%) cases and absent in 40 (50.0%) cases. Assessment of the Ki-67 proliferation index showed low proliferative activity (<14%) in 28 (35.0%) tumours, whereas 52 (65.0%) exhibited high proliferative activity (≥14%).
The results of NRP2 and SNAIL/SLUG there expression and correlation with the clinicopathological characteristics are presented in tables, followed by selected figures illustrating the principal findings as shown in Table:1-5 and Figure 1-2 . High NRP2 expression was present in 22 cases (27.5%), while high SNAIL/SLUG expression was present in 32 cases (40.0%). Both markers were highly expressed in 16 tumors (20.0%). Among tumors with high NRP2 expression, 72.7% also had high SNAIL/SLUG expression compared with 27.6% among tumors with low NRP2 expression. This co-expression was statistically significant (Fisher's exact p<0.001; OR=7.00). Numerical NRP2 and SNAIL/SLUG scores showed a significant positive correlation (Spearman ρ=0.372, p<0.001) as shown in Table:1,2.

Table 1. Distribution of NRP2 and SNAIL/SLUG expression

Marker	Expression category	n (%)
NRP2	Low expression	58 (72.5%)
NRP2	High expression	22 (27.5%)
SNAIL/SLUG	Low expression	48 (60.0%)
SNAIL/SLUG	High expression	32 (40.0%)

Table 2. Co-expression and correlation between NRP2 and SNAIL/SLUG

NRP2 / analysis	Low SNAIL/SLUG	High SNAIL/SLUG	p value
Low NRP2	42 (72.4%)	16 (27.6%)	0.000339
High NRP2	6 (27.3%)	16 (72.7%)	
Numerical score correlation	Spearman ρ	0.372	0.000691
Odds ratio for high/high co-expression	OR	7.00	

The strongest finding was the relationship between NRP2 and SNAIL/SLUG. High expression of both markers occurred together significantly more often than expected, and their numerical scores were positively correlated. SNAIL/SLUG expression was significantly associated with ER status (p=0.011), with high expression more frequent in ER-negative tumors. PR-negative tumors also showed a higher proportion of high SNAIL/SLUG expression, although this did not reach statistical significance (p=0.069). NRP2 high expression was more frequent in node-positive tumors than node-negative tumors, but the association was not statistically significant (p=0.210). Both markers showed higher expression proportions in biologically aggressive categories, particularly triple-negative tumors, but the molecular-subtype associations did not reach the conventional p<0.05 threshold in this sample. As shown in Table:3-5.

Table 3. Association of NRP2 and SNAIL/SLUG expression with clinicopathological factors

Variable	Category	High NRP2 n/N (%)	p	High SNAIL/SLUG n/N (%)	p
Age group	≤50 years	7/33 (21.2%)	0.322	12/33 (36.4%)	0.647
	>50 years	15/47 (31.9%)		20/47 (42.6%)	
Tumor size group	≤2 cm	0/1 (0.0%)	0.327	0/1 (0.0%)	0.465
	2.1–5 cm	9/42 (21.4%)		15/42 (35.7%)	
	>5 cm	13/37 (35.1%)		17/37 (45.9%)	
Grade	Grade I	9/44 (20.5%)	0.290	14/44 (31.8%)	0.227
	Grade II	11/31 (35.5%)		15/31 (48.4%)	
	Grade III	2/5 (40.0%)		3/5 (60.0%)	
Stage group	Stage I	0/2 (0.0%)	0.290	0/2 (0.0%)	0.106
	Stage II	9/41 (22.0%)		13/41 (31.7%)	
	Stage III	13/37 (35.1%)		19/37 (51.4%)	
Node status	Negative	8/40 (20.0%)	0.210	16/40 (40.0%)	1.000
	Positive	14/40 (35.0%)		16/40 (40.0%)	
Ki-67	Low (<14%)	7/28 (25.0%)	0.797	8/28 (28.6%)	0.155
	High (≥14%)	15/52 (28.8%)		24/52 (46.2%)	

Table 4. Association of NRP2 and SNAIL/SLUG expression with hormonal biomarker status

Biomarker	Status	High NRP2 n/N (%)	p	High SNAIL/SLUG n/N (%)	p
ER status	Negative	13/35 (37.1%)	0.130	20/35 (57.1%)	0.011
	Positive	9/45 (20.0%)		12/45 (26.7%)	
PR status	Negative	13/37 (35.1%)	0.210	19/37 (51.4%)	0.069
	Positive	9/43 (20.9%)		13/43 (30.2%)	
HER2 status	Negative	14/43 (32.6%)	0.486	15/43 (34.9%)	0.437
	Equivocal	3/17 (17.6%)		9/17 (52.9%)	
	Positive	5/20 (25.0%)		8/20 (40.0%)	

Table 5. NRP2 and SNAIL/SLUG expression according to molecular subtype (n=63)

Molecular subtype	n	High NRP2 n (%)	High SNAIL/SLUG n (%)
Luminal A	20	5 (25.0%)	4 (20.0%)
Luminal B	23	4 (17.4%)	8 (34.8%)
HER2-enriched	4	1 (25.0%)	1 (25.0%)
Triple-negative	16	9 (56.2%)	10 (62.5%)

Overall association with molecular subtype: NRP2 p=0.064; SNAIL/SLUG p=0.064. HER2-equivocal tumors (n=17) were excluded from subtype classification.

Figures

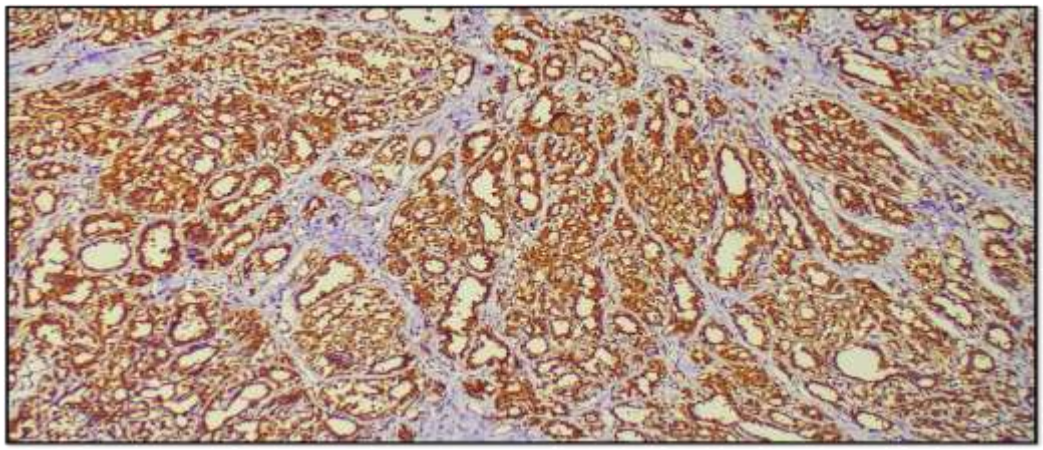

Figure 1. Immunohistochemical staining for SNAIL/SLUG showing high expression (100×).

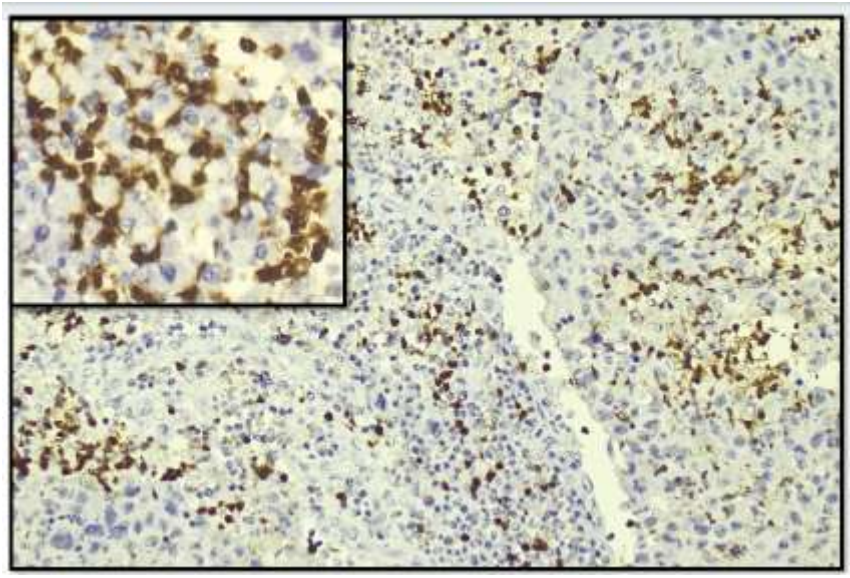

Figure 2. Immunohistochemical staining for NRP2 showing membranous positivity (100×); inset showing membranous staining at higher magnification (400×).

DISCUSSION

The present study, NRP2 was highly expressed in 27.5% of tumors ,while SNAIL/SLUG expression was high in 40.0%(Fig:1). The most important observation was their significant co-expression: tumors with high NRP2 (Fig:2) were substantially more likely to show high SNAIL/SLUG expression (OR=7.0), with a positive numerical correlation (ρ=0.372).as seen in Table: 1.This finding supports a biologically linked role for NRP2 and EMT-associated transcriptional activity.NRP2 is a multifunctional transmembrane receptor involved in VEGF signalling, lymphangiogenesis, cell migration, tumor initiation and metastatic progression. Yasuoka et al. reported NRP2 expression in 53.1% of invasive breast carcinomas and demonstrated significant associations with lymph-node metastasis, VEGF-C and CXCR4 expression, together with poorer overall survival.[11] Similarly, Zhao et al. showed that NRP2 promotes malignant behavior in triple-negative breast cancer, whereas NRP2 suppression reduced cellular proliferation.[12] Kumar et al. demonstrated that NRP2 promotes tumour cell survival and therapeutic resistance in triple-negative breast cancer, further supporting the role of NRP2 in aggressive tumour behaviour. [13]

Although high NRP2 expression was more frequent in node-positive tumors in the present study (35.0% vs 20.0%), this association was not statistically significant (p=0.210). This may reflect the relatively small sample size as seen in Table:2. Nevertheless, the direction of association agrees with Yasuoka et al., who reported a significant relationship between NRP2 and lymph-node metastasis.[11] . Dhupar et al. additionally highlighted the involvement of NRP2 in tumor-associated macrophage biology, supporting its broader role within the breast-cancer microenvironment.[14].

In this study, high NRP2 and SNAIL/SLUG expression demonstrated increasing trends with larger tumor size, higher histological grade, advanced TNM stage, and higher Ki-67 index; however, none of these associations reached statistical significance(Table: 3).Similar trends have been reported by Yasuoka et al. for NRP2[11] and by Abd ElMoneim and Zaghloul et al for Snail, although those investigators observed statistically significant correlations with aggressive clinicopathological features.[15]

Furthermore Zhang et al. A systematic review and meta-analysis reported no significant association between Slug expression and patient age, tumor size, or histological grade, findings that are consistent with the present study Table3.[5].Similarly study done by Jacob S et al .evaluating EMT markers in breast carcinoma also suggested that the absence of statistical significance for some variables may be attributable to limited sample size, despite biologically meaningful trends.[9].SNAIL/SLUG showed a significant association with ER negativity (57.1% vs 26.7%, p=0.011) as shown in Table:4. This finding is consistent with Bai JW et al., who demonstrated an inverse relationship between SLUG and ERα expression in breast cancer.[16] A large tissue-microarray study of 1043 breast cancers by Muenst S. similarly found that SNAIL expression was strongly associated with hormone-receptor negativity, higher grade, advanced stage and basal-like disease.[17] A meta-analysis by Zhang et al. confirmed that increased SLUG expression is associated with higher TNM stage, lymph-node metastasis and ER deficiency[5].Martin et al.also reported increased SLUG expression with adverse prognostic characteristics and metastatic disease.[18]. It should be noted that several previous studies evaluated SNAIL or SLUG individually, whereas the present study assessed combined SNAIL/SLUG immunohistochemical expression; therefore, direct comparison should be interpreted with caution.The present study also demonstrated the highest NRP2 (56.2%) and SNAIL/SLUG (62.5%) expression in triple-negative tumors, although subtype associations did not reach statistical significance (p=0.064)as seen in Table 5. This trend is biologically plausible, as Wu et al. demonstrated close involvement of SLUG with EMT and BRCA1-related pathways in triple-negative breast cancer.[19] Overall, these findings suggest that coordinated NRP2–SNAIL/SLUG activation may contribute to an aggressive, hormone-receptor-negative phenotype, warranting validation in larger cohorts.The findings of Nakayama et al. further support the biological relevance of neuropilin-2 (NRP2) in breast cancer metastasis, demonstrating that Semaphorin 3F inhibits breast cancer metastasis through modulation of the Akt–mTOR and TGF-β signaling pathways

via NRP2. In the present study, NRP2 expression was observed in 27.5% of tumors and was higher in node-positive cases than in node-negative cases (35% vs. 20%); however, this difference did not reach statistical significance.[20]

CONCLUSION

The present study demonstrates that NRP2 and SNAIL/SLUG are frequently expressed in breast carcinoma and show a significant positive relationship. Their significant co-expression suggests a potential biological link between NRP2-mediated signaling and epithelial–mesenchymal transition. SNAIL/SLUG expression was significantly higher in ER-negative tumors, while both markers demonstrated increased expression in biologically aggressive subgroups, particularly triple-negative breast cancer. Although associations with lymph-node status, histological grade, stage, and molecular subtype did not reach statistical significance, the observed trends support their potential involvement in tumor aggressiveness. Larger prospective studies are warranted to validate these findings and clarify their prognostic and therapeutic significance.

Limitations: The present study was limited by its relatively small sample size and single-centre retrospective design, which may have reduced the statistical power and generalizability of the findings. The lack of follow-up data precluded survival analysis, while absence of confirmatory ISH testing for HER2-equivocal cases and molecular validation of NRP2 and SNAIL/SLUG expression represent additional limitations.

DECLARATIONS:

Conflicts of interest: The authors declare that there are no conflicts of interest.

Consent to participate: The requirement for informed consent was waived by the Institutional Ethics Committee due to the retrospective nature of the study and use of archival specimens.

Consent for publication: Consent for publication was obtained.

Authors' contributions: All authors contributed equally to the study and approved the final manuscript.

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