RESEARCH ARTICLE

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The Expression of Semaphorin 3G (SEMA3G) Protein in Breast Cancer Tissue by the Immunohistochemistry Method

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Abstract

Objective: Breast cancer is a major health issue for women worldwide, with high incidence rates and as one of the primary causes of mortality among women. However, molecular studies related to the development and spread of breast cancer are still not well understood. According to research reports, the *Semaphorin 3G (SEMA3G)* gene significantly contributes to the growth and dissemination of cancer cells. The aim of this study was to compare the expression of the SEMA3G protein found in healthy breast tissue versus breast cancer patient tissue and to investigate the correlation between SEMA3G protein expression and the spread of breast cancer cells to axillary lymph nodes, as well as other clinical data, using immunohistochemistry. **Results:** From the study of 88 breast tissue samples, it was found that *SEMA3G* levels, with an immunohistochemistry (IHC) index > 4, were significantly higher in breast cancer tissue compared to normal breast tissue (P-value < 0.0001, OR (95% CI) = 8.565 to +infinity). Moreover, *SEMA3G* protein expression (IHC index > 6) was detected at elevated levels in breast cancer tissue that had spread to axillary lymph nodes compared with breast cancer tissue without lymph node metastasis (P-value = 0.038, OR (95% CI) = 4.41 (1.19–13.81)). Additionally, the study found a positive correlation between high SEMA3G protein expression and *HER2*-positive breast cancer. **Conclusions:** Higher *SEMA3G* expression was significantly associated with breast cancer presence, axillary lymph node metastasis, and *HER2* positivity, indicating that SEMA3G may play a role in tumor aggressiveness and could serve as a potential marker for disease progression, with potential implications for clinical decision-making in treatment strategies.

Keywords: Semaphorin 3G- SEMA3G- Breast Cancer- Axillary Lymph Node- HER2

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Introduction

Breast cancer is a global public health issue that leads to a significant loss of life and substantial medical treatment costs. In 2018, there were 2.09 million new cases, accounting for 24.2% of all cancers, and it is the most common cancer in women [1]. In Thailand, according to hospital cancer registries, breast cancer is one of the most prevalent cancers among women, with 780 new cases reported in 2017 [2]. It is most commonly found in women aged 40 and above [3]. The most common type of breast cancer in Thai women is ductal carcinoma [4]. According to a report from Songklanagarind Hospital in southern Thailand, breast cancer is the most frequently diagnosed cancer in women, with an incidence of 671 cases in 2017 [5].

To classify breast cancer patients for prognostication and treatment decisions, clinical and pathological data are used, with prognostic markers commonly employed in daily practice, such as patient age, tumor size, lymph node metastasis, nuclear grade, and the expression of hormone receptors like estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [6]. Among these markers, axillary lymph node status is considered the most critical indicator for prognostic breast cancer [7], as the spread of cancer cells to the axillary lymph nodes is crucial for prognosis and determining subsequent treatment options [8]. For accurate staging of the disease, which aids in planning treatment and predicting outcomes, doctors must perform surgery to remove axillary lymph nodes to check for metastasis. Therefore, if it is possible to predict that breast cancer cells have the potential to spread to the axillary lymph nodes by analyzing the expression of proteins in the initial breast cancer tissue sample, it could potentially eliminate the need for further lymph node

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surgery to detect additional cancer cells, thereby reducing unnecessary surgery.

Semaphorin 3G (SEMA3G), a member of the class 3 semaphorin family, has been reported to function predominantly as a tumor suppressor in several cancer types [9]. The expression of SEMA3G can inhibit cell invasion and migration. Research has shown that overexpression of SEMA3G can suppress the invasion and migration of cells, as these processes are key mechanisms in cancer metastasis. Therefore, proteins involved in these processes are considered highly significant [10]. In contrast, over-expression of SEMA3Gb results in an abnormal cranial neural crest cells (NCCs) migration phenotype in zebrafish [11]. However, emerging evidence suggests that the role of SEMA3G may be context-dependent, with its expression and function varying across tissue types and tumor microenvironments [12]. In normal physiology, SEMA3G is involved in vascular patterning, axon guidance, and immune regulation [13, 14].

We previously demonstrated the overexpression of SEMA3G in breast cancer tissues compared to normal counterparts using real-time quantitative PCR [15]. The research team intends to expand the study to examine protein expression levels, which will be assessed using immunohistochemistry. The study aims to compare the protein expression of SEMA3G in breast cancer cells to its expression in normal tissue. Investigation will include 29 samples of cancer tissue with axillary lymph node metastasis (Axillary node positive), 29 samples without lymph node metastasis (Axillary node negative), and 30 normal breast tissue samples. Statistical analysis will be performed to determine whether the expression of the SEMA3G protein can serve as a prognostic marker for the spread of cancer cells to the axillary lymph nodes. This could be valuable for planning treatment and predicting outcomes in breast cancer patients.

Materials and Methods

Human subjects and tissue specimens

Tissue samples, including invasive ductal breast cancer and normal breast tissues, were collected from the Department of Anatomical Pathology, Hatyai Hospital, Songkhla Province, between January 2020 and December 2021. These samples were obtained from female patients who had not undergone radiotherapy or neoadjuvant therapies prior to tissue collection. Patient characteristics included age at diagnosis, type of tissue, tumor grade, and regional lymph node status. Histological grading of breast cancer was performed using standard criteria: Grade I (well-differentiated tumors), Grade II (moderately differentiated tumors), and Grade III (poorly differentiated tumors). This study was conducted under a protocol approved by the Ethics Committee of Hatyai Hospital (HYH EC 114-64-02) and the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects (WUEC-21-328-01). Informed consent was not obtained; however, all patient records and information were anonymized and de-identified prior to analysis. Patient names and Hospital Numbers (HN) were not disclosed to ensure confidentiality.

Inclusion and Exclusion Criteria

Only patients with invasive ductal carcinoma (IDC) of the breast, who had not received prior chemotherapy or radiotherapy, were included. The tissue samples were categorized as follows: 30 normal breast tissues, 29 samples with axillary node-negative breast cancer, and 29 samples with axillary node-positive breast cancer.

Immunohistochemistry (IHC)

The IHC protocol for detecting SEMA3G protein expression followed the established procedure, as described by Ramos-Vara [16], with modifications as detailed below.

Tissue blocks were sectioned at 5 μm and mounted on positively charged slides. Sections were deparaffinized with xylene and rehydrated through a graded ethanol series (100%, 80%, 70%). Antigen retrieval was performed in 0.01 M sodium citrate buffer (pH 6.0) using a pressure cooker at 125°C for 4 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 30 minutes in the dark at room temperature. Non-specific binding was minimized by incubating with normal serum for 30 minutes in a humid chamber. Slides were then incubated overnight at room temperature with a rabbit polyclonal anti-SEMA3G primary antibody (1:100 dilution). The next day, sections were incubated with a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:250 dilution) for 30 minutes, followed by visualization with 3,3'-diaminobenzidine (DAB) substrate. Nuclei were counterstained with Mayer's hematoxylin, then slides were dehydrated, cleared in xylene, and mounted. Evaluation of *SEMA3G* expression was performed independently by two blinded researchers using light microscopy at 100× and 400× magnification across 10–20 representative fields per slide. The percentage of positively stained cells was scored as follows: 0% (negative), 1–25% (+1), 26–50% (+2), and >50% (+3). Staining intensity was scored as weak (1), moderate (2), or strong (3). The IHC index for each sample was calculated by multiplying the intensity score by the percentage score.

Statistical analysis

Descriptive statistics (mean ± standard deviation for continuous variables; frequencies and percentages for categorical variables) were used to summarize patient demographics and clinicopathological characteristics. Protein expression was calculated as the Immunohistochemistry (IHC) index = % Positive cells × intensity. The expression of the SEMA3G protein in normal individuals versus breast cancer patients was determined using Student's t-test for comparisons between two independent groups. The association between *SEMA3G* expression and clinicopathological variables was analyzed using Fisher's exact test. Binary logistic regression was used to assess the relationship between *SEMA3G* expression and lymph node metastasis, providing odds ratios (ORs) with 95% confidence intervals (CIs). A two-sided P value < 0.05 was considered statistically significant. All analyses were conducted using Statistical Package for the Social Sciences (SPSS, Inc.,

Chicago, IL, USA).

Results

Characteristics of the tissue samples studied

In this study, a total of 88 breast tissue samples were analyzed, consisting of 30 normal tissue samples (34.1%) and 58 breast cancer tissue samples (65.9%). Among the breast cancer samples, 29 samples (50.0%) had spread to the axillary lymph nodes, while 29 samples (50.0%) did not show lymph node metastasis. The average age of all patients was 45.1 ± 15.4 years, with an average tumor dimension of 3.46 ± 1.86 cm. The majority of the breast cancer samples were categorized as Grade 1 (2 samples), Grade 2 (30 samples, 51.7%), and Grade 3 (25 samples, 43.1%), with 1 sample (1.7%) lacking clinical data. In the breast cancer tissue samples, estrogen receptor (ER) expression was observed as follows: no ER expression in 14% of cases and ER expression present in 42%. Progesterone receptor (PR) expression showed no PR expression in 50% of cases, and PR expression was present in 46.5%. For human epidermal growth factor receptor 2 (HER2) expression, no *HER2* expression was identified in 51.7% of cases, while *HER2* expression was present in 44.8%. In summary, the breast cancer cohort was relatively balanced in terms of lymph node status and showed heterogeneity in receptor status and tumor grade. These results are summarized in Table 1.

Table 1. Demographic and Clinicopathological Characteristics of Patients (n=88)

Variables	N (%)
Age; years (mean±SD)	45.1 ± 15.4
Type of breast	
Normal	30 (34.1)
Cancer	58 (65.9)
Tumor size; cm (mean±SD)	3.46 ± 1.86
Tumor grade	
Grade I	2 (3.5)
Grade II	30 (51.7)
Grade III	25 (43.1)
No data	1 (1.7)
Lymph node metastasis	
No	29 (50.0)
Yes	29 (50.0)
Estrogen receptor	
Negative	14 (24.1)
Positive	42 (72.4)
No data	2 (3.5)
Progesterone receptor	
Negative	29 (50.0)
Positive	27 (46.5)
No data	2 (3.5)
Human Epidermal Growth Factor Receptor 2	
Negative	30 (51.7)
Positive	26 (44.8)
No data	2 (3.5)

*, Tumor size based on longest diameter; * N, Number of individuals

Expression of SEMA3G Protein

The SEMA3G protein was expressed in all types of breast tissue samples, including both normal and cancerous tissues, as illustrated in Figure 1 and Table 2. The experimental outcomes revealed the following: In normal breast tissue, the mode immunohistochemistry index (IHC index) was 0 (15/30 samples, 50.0%). In breast cancer tissue with absent lymph node metastasis, the mode IHC index was 6 (11/29 samples, 37.9%). In tumor tissue of the breast having axillary lymph node metastasis, the mode IHC index was 9 (12/29 samples, 41.4%). These results suggest a progressive increase in *SEMA3G* expression from normal tissue to metastatic breast cancer, implying a possible association with tumor progression.

Expression of SEMA3G Protein and Prognostic Factors in Breast Cancer

To test whether the expression of SEMA3G protein could serve as an indicator of breast cancer development and its severity, we used Fisher's exact test to analyze the relationship between *SEMA3G* expression and various characteristics of breast tissue, as well as the cancer's metastatic properties. The analysis used cut-off values of IHC index > 4 or ≤ 4 and IHC index > 6 or ≤ 6 (with

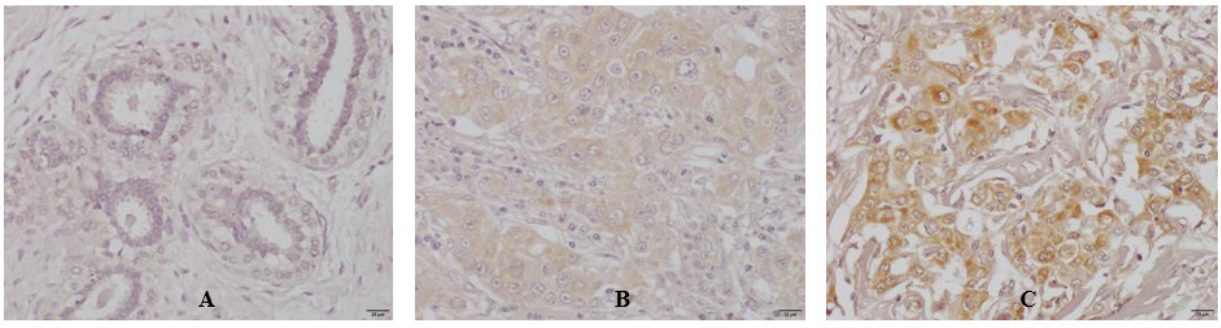

Figure 1. The expression of *SEMA3G* in Normal Breast Tissue (A), breast tissue with axillary node-negative status (B), and breast tissue with axillary node-positive status (C) (40X magnification). The percentage of positively stained cells was graded as 0% (score 0), 1–25% (score 1), 26–50% (score 2), and >50% (score 3), while staining intensity was graded as weak (score 1), moderate (score 2), or strong (score 3). The IHC index was obtained by multiplying the percentage score by the intensity score.

Table 2. *SEMA3G* Expression in Breast Tissues

IHC index	Types of breast tissues			Total (%)
	Normal (%)	Axillary nodes negative (%)	Axillary nodes positive (%)	
0	15 (50.0)	2 (6.9)	3 (10.3)	20 (22.7)
1	3 (10.0)	2 (6.9)	1 (3.4)	6 (6.8)
2	4 (13.3)	1 (3.4)	1 (3.4)	6 (6.8)
3	7 (23.3)	7 (24.1)	4 (13.8)	18 (20.5)
4	1 (3.3)	2 (6.9)	4 (13.8)	7 (7.9)
6	0 (0)	11 (37.9)	4 (13.8)	15 (17.1)
9	0 (0)	4 (13.8)	12 (41.4)	16 (18.2)

*IHC index refers to the Immunohistochemistry index

average IHC index values of 3.79 and 5.14, respectively). The analysis showed the following: The expression of SEMA3G with IHC index > 4 was significantly higher in breast cancer tissue compared to normal breast tissue (P-value < 0.0001, OR (95% CI) = 8.565 to +infinity). The IHC index > 6 was significantly more common in breast cancer tissue with metastasis to the axillary lymph nodes compared to those without lymph node metastasis (P-value = 0.038, OR (95% CI) = 4.41 (1.19-13.81)), as shown in Table 3.

Furthermore, the study found a positive correlation between *SEMA3G* expression (IHC index > 6) and the expression of *HER2* in breast cancer tissue. A positive correlation between *SEMA3G* expression and *HER2* receptor positivity was observed (P = 0.02). The analysis revealed that *SEMA3G* expression was significantly higher in HER2-positive tumors compared to HER2-negative tumors. However, no statistically significant differences were found between *SEMA3G* expression (IHC index > 6 or ≤ 6) and other prognostic markers, including: Age, Tumor size, Tumor stage, Tumor grade, Estrogen receptor (ER), Progesterone receptor (PR), *Ki-67* proliferative index (cell proliferation marker). Overall, higher *SEMA3G* expression was significantly associated with breast cancer presence, lymph node metastasis, and *HER2* positivity, suggesting a potential role of *SEMA3G* in tumor progression and aggressiveness. These results are summarized in Table 4.

Discussion

From the study on the expression of Semaphorin3G (SEMA3G) protein in breast cancer using immunohistochemistry (IHC), utilizing breast tissue samples from patients who had never received treatment, preserved in paraffin-embedded blocks at the Department of Anatomical Pathology, Hat Yai Hospital, the following conclusions were made: The study on the expression of SEMA3G protein revealed that the IHC index was measured in 88 breast tissue samples: 30 normal tissue samples and 58 breast cancer samples, with 29 samples showing no lymph node metastasis and 29 samples showing metastasis to axillary lymph nodes. The results showed the average IHC index was 1.2 (mode = 0) for normal breast tissue, 4.6 (mode = 6) for breast cancer samples with absent lymph node metastasis, and 5.6 (mode = 9) for breast cancer tissue with the presence of lymph node metastasis. This indicates that *SEMA3G* expression was elevated in breast cancer tissue compared to normal tissue, with the highest expression found in breast cancer tissue with lymph node metastasis. When comparing the expression of *SEMA3G* in breast cancer tissue and normal tissue, the study found a statistically significant increase in *SEMA3G* expression in breast cancer tissue. Furthermore, when comparing the expression of *SEMA3G* in breast cancer tissue presence and absence lymph node metastasis, the expression was significantly higher in the samples with metastasis. These IHC-based findings are consistent with molecular data reported by Kotepui et al. [15], which assessed *SEMA3G* mRNA levels using quantitative real-time RT-PCR and found elevated expression in tumor tissues compared to adjacent normal tissues. However, these results contradict previous research by Kigel B et al. [17] and Karayan-Tapon L et al. [18], which stated that

Table 3. IHC Index of SEMA3G in Breast Tissues, Cut-off (> 4 and ≤ 4) and (> 6 and ≤ 6)

SEMA3G expression	Breast Tissues		P-value	OR (95% CI)
	Cancer (%)	Normal (%)		
IHC index				
> 4	27 (46.6)	0 (0.0)	<0.0001	8.57 to +infinity
≤ 4	31 (53.4)	30 (100.0)		
	Axillary lymph nodes			
	Positive (%)	Negative (%)		
IHC index				
> 6	12 (41.4)	4 (13.8)	0.038	4.41 (1.19–13.81)
≤ 6	17 (58.6)	25 (86.2)		

*, IHC index refers to the Immunohistochemistry index; OR, refers to Odds Ratio; CI, refers to Confidence Interval; * P-value by Fisher's exact test

Table 4. Univariate Analysis of Low and High *SEMA3G* Expression in 58 Breast Cancer Patients

Variable	N	*SEMA3G* expression		P-value
		Low (≤ 6)	High (> 6)	
Age				
≤ 45 yrs	16	13	3	0.515
> 45 yrs	42	29	13	
Staging				0.186
I	3	3	0	
IIA	28	23	5	
IIB	22	13	9	
IIIA	5	3	2	
Tumor size*				0.109
< 3.5 cm	42	33	9	
> 3.5 cm	16	9	7	
Tumor grade				0.466
I	3	3	0	
II	30	22	8	
III	24	16	8	
Lymph node metastasis				0.038
No	29	25	4	
Yes	29	17	12	
ER				> 0.999
Negative	14	10	4	
Positive	42	31	11	
PR				0.233
Negative	29	19	10	
Positive	27	22	5	
HER2				0.018
Negative	30	26	4	
Positive	26	15	11	
Ki-67 proliferative index (%)				0.314
< 48	26	21	5	
> 48	18	12	6	

* Tumor size based on longest diameter; * P-value by Fisher's exact test; * SEMA3G, Semaphorin 3G; IHC, Immunohistochemistry; OR, Odds Ratio; CI, Confidence Interval; ER, Estrogen Receptor; PR,Progesterone Receptor; HER2, Human Epidermal Growth Factor Receptor 2; cm , Centimeter; yrs, Years; N, Sample Size; *Ki-67*, A marker of cell proliferation.

higher *SEMA3G* expression can inhibit tumor growth and angiogenesis in breast cancer, melanoma, and brain cancer cells. The differing results may be due to variations in experimental models, sample sizes, or cancer types. It is also possible that *SEMA3G*'s role in cancer metastasis is context-dependent, influenced by factors such as tumor microenvironment, cancer stage, or the presence of specific molecular markers like HER2.

In evaluating the correlation between the expression of SEMA3G protein (IHC index > 6) and clinical factors such as breast cancer metastasis, a positive correlation was found between *SEMA3G* expression and metastasis to the axillary lymph nodes. This finding contradicts the study by Zhou X et al. [10] on brain cancer, where *SEMA3G* expression was illustrated to restrict the movement and spread of cancer cells. It also contrasts with the report by Zhang X et al. [19], which showed high levels of *SEMA3G* in stromal cells. Additionally, the relationship between *SEMA3G* expression (IHC index > 6) and *HER2* receptor expression in breast cancer tissue was found to be positively correlated. Other reports showed that endothelial cells (ECs)-derived *SEMA3G* under diabetic condition activated Yes-Associated Protein (YAP) and promoted Human Aortic Smooth Muscle Cells (HASMCs) proliferation and migration via Nrp2/PlexinA1 [20]. Interestingly, *SEMA3G* was widely upregulated in diverse human cancers, and its expression was positively correlated with tumor progression. *SEMA3G* acted as a ligand that inhibited the activation and functionality of T cells [21]. However, no studies have yet reported a direct relationship between *SEMA3G* and HER2, although there is evidence that increased *HER2* expression can stimulate cancer cell growth, movement, and metastasis, both in vitro and in vivo [22-28].

While our study provides valuable insights into the role of *SEMA3G* in breast cancer metastasis, the sample size remains a limitation. Larger, multi-center studies are needed to validate these findings and to explore potential therapeutic strategies targeting *SEMA3G* in breast cancer. Additionally, mechanistic studies involving cell culture and animal models will help clarify the role of *SEMA3G* in tumor progression and its interaction with other molecular pathways, including HER2.

In conclusion, this study demonstrates that *SEMA3G* protein expression is significantly elevated in breast cancer tissues, particularly in those with axillary lymph node metastasis and HER2-positive status. These findings suggest that *SEMA3G* may be involved in pathways promoting tumor aggressiveness, including those mediated by *HER2* signaling. The correlation between *SEMA3G* overexpression and *HER2* expression raises the possibility that *SEMA3G* could contribute to or reflect HER2-driven oncogenic activity. Therefore, *SEMA3G* may serve as a prognostic biomarker and potential therapeutic target in HER2-positive breast cancer, warranting further mechanistic studies.

Author Contribution Statement

DP and KUK conceptualized the study and drafted the manuscript. KUK and AS conducted the experiments, while DP and CC collected the data. KUK, DP, and MK were responsible for data extraction and analysis. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

This study followed the Declaration of Helsinki and was conducted under a protocol approved by the Ethics Committee of Hatyai Hospital (HYH EC 114-64-02) and the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects at Walailak University (WUEC-21-328-01). Consent to participate was waived by the ethics committees, as leftover specimens and retrospective samples were used.

References

1. GLOBOCAN. GLOBOCAN 2018 database [Internet]. Lyon: International Agency for Research on Cancer; c2018 [cited 2025 Aug 4]. Available from: http://gco.iarc.fr/
2. National Cancer Institute. Hospital-based cancer registry 2560 [Internet]. Bangkok: NCI Thailand; 2017 [cited 2025 Aug 4]. Available from: https://www.nci.go.th/th/File_download/Nci%20Cancer%20Registry/HOSPITAL-BASED%202016%20Revise%204%20Final.pdf
3. Kotepui M, Chupeerach C. Age distribution of breast cancer from a Thailand population-based cancer registry. Asian Pac J Cancer Prev. 2013;14(6):3815-7. doi: 10.7314/APJCP.2013.14.6.3815.
4. Wilailak S, Lertchaipattanakul N. The epidemiologic status of gynecologic cancer in Thailand. J Gynecol Oncol. 2016;27(6):e65. doi:10.3802/jgo.2016.27.e65
5. Prechawittayakul P. Hospital based tumor registry. Songklanagarind Hospital: Songklanagarind Hospital; 2019.
6. Takahashi Y, Iwai M, Kawai T, Arakawa A, Takano M, Ogata H, et al. Aberrant expression of tumor suppressors CADM1 and 4.1B in invasive lesions of primary breast cancer. Breast Cancer. 2012;19(3):242–52. doi: 10.1007/s12282-011-0330-3.
7. Fisher B, Bauer M, Wickerham DL, Redmond CK, Fisher ER, Cruz AB Jr, et al. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP update. Cancer. 1983;52(9):1551–7. doi: 10.1002/1097-0142(19831101)52:9<1551::aid-cncr2820520902>3.0.co;2-3.
8. Rosen PR, Groshen S, Saigo PE, Kinne DW, Hellman S. A long-term follow-up study of survival in stage I (T1N0M0) and stage II (T1N1M0) breast carcinoma. J Clin Oncol. 1989;7(3):355–66. doi: 10.1200/JCO.1989.7.3.355.
9. Nasarre P, Gemmill RM, Drabkin HA. The emerging role of class-3 semaphorins and their neuropilin receptors in oncology. Onco Targets Ther. 2014;7:1663-87. doi:10.2147/OTT.S45609
10. Zhou X, Ma L, Li J, Gu J, Shi Q, Yu R. Effects of SEMA3G on migration and invasion of glioma cells. Oncol Rep. 2012;28(1):269-75. doi:10.3892/or.2012.1796
11. Yu HH, Moens CB. Semaphorin signaling guides cranial neural crest cell migration in zebrafish. Dev Biol. 2005;280(2):373-85. doi:10.1016/j.ydbio.2005.01.029
12. Casazza A, Laoui D, Wenes M, Rizzolio S, Bassani N, Mambretti M, et al. Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. Cancer Cell. 2013;24(6):695-709. doi:10.1016/j.ccr.2013.11.007
13. Püschel AW. Semaphorins: Receptors and intracellular signaling. Semin Cell Dev Biol. 2007;18(1):63-71. doi:10.1016/j.semcdb.2006.11.002
14. Suzuki K, Okuno T, Yamamoto M, Pasterkamp RJ, Takegahara N, Takamatsu H, et al. Semaphorin 3G is a novel immunomodulatory molecule that suppresses T-cell activation and is involved in autoimmune pathogenesis. Immunol Lett. 2007;112(1):55-62. doi:10.1016/j.imlet.2007.07.004
15. Kotepui M, Thawornkuno C, Chavalitshewinkoon-Petmitr P, Punyarit P, Petmitr S. Quantitative real-time RT-PCR of ITGA7, SVEP1, TNS1, LPHN3, SEMA3G, KLB and MMP13 mRNA expression in breast cancer. Asian Pac J Cancer Prev. 2012;13(11):5879-82. doi: 10.7314/APJCP.2012.13.11.5879.
16. Ramos-Vara JA. Technical aspects of immunohistochemistry. Vet Pathol. 2005;42(4):405–26. doi: 10.1354/vp.42-4-405.
17. Kigel B, Varshavsky A, Kessler O, Neufeld G. Successful inhibition of tumor development by specific class-3 semaphorins is associated with expression of appropriate semaphorin receptors by tumor cells. PLoS One. 2008;3(9):e3287. doi: 10.1371/journal.pone.0003287.
18. Karayan-Tapon L, Wager M, Guilhot J, Levillain P, Karayan-Tapon I, Moreau-Fauvarque C, et al. Semaphorin, neuropilin and VEGF expression in glial tumours: SEMA3G, a prognostic marker? Br J Cancer. 2008;99(7):1153-60. doi:10.1038/sj.bjc.6604653
19. Zhang X, Klamer B, Li J, Fernandez S, Li L. A pan-cancer study of class-3 semaphorins as therapeutic targets in cancer. BMC Med Genomics. 2020;13(Suppl 5):45. Published 2020 Apr 3. doi:10.1186/s12920-020-0682-5
20. Luo XY, Fu X, Liu F, Luo JY, Chen AF. Sema3G activates YAP and promotes VSMCs proliferation and migration via Nrp2/PlexinA1. Cell Signal. 2023;105:110613. doi:10.1016/j.cellsig.2023.110613
21. Chi H, Deng S, Xu K, et al. SEMA3G-NRP1 signaling functions as an immune checkpoint that enables tumor immune evasion by impairing T cell cytotoxicity. Cancer Res. Published online 2024 Dec 9. doi:10.1158/0008-5472.CAN-24-2223
22. Meric F, Hung MC, Hortobagyi GN, Hunt KK. HER2/neu in the management of invasive breast cancer. J Am Coll Surg. 2002;194(4):488–501. doi: 10.1016/S1072-7515(02)01121-3.
23. Hung MC, Lau YK. Basic science of HER-2/neu: a review. Semin Oncol. 1999;26(4 Suppl 12):51–9. doi: 10.1016/S0093-7754(99)80012-4.
24. 24. Li YM, Pan Y, Wei Y, Cheng X, Zhou BP, Tan M, et al. Upregulation of CXCR4 is essential for HER2-mediated tumor metastasis. Cancer Cell. 2004;6(5):459–69. doi: 10.1016/j.ccr.2004.09.027.
25. Subbaramaiah K, Howe LR, Port ER, Brogi E, Fishman J, Soslow R, et al. HER-2/neu status is a determinant of mammary aromatase activity in vivo: evidence for a cyclooxygenase-2-dependent mechanism. Cancer Res. 2006;66(10):5504–11. doi: 10.1158/0008-5472.CAN-05-4715.
26. Tan M, Yao J, Yu D. Overexpression of the c-erbB-2 gene enhanced intrinsic metastasis potential in human breast cancer cells without increasing their transformation abilities. Cancer Res. 1997;57(6):1199–205. doi: 10.1158/0008-5472.CAN-57-6-1199.
27. Suda Y, Aizawa S, Furuta Y, et al. Induction of a variety of tumors by c-erbB2 and clonal nature of lymphomas even with the mutated gene (Val659→Glu659). EMBO J. 1990;9(1):181–90. doi: 10.1002/j.1460-2075.1990.tb08094.x.
28. Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ. Expression of the neu protooncogene in the

mammary epithelium of transgenic mice induces metastatic disease. Proc Natl Acad Sci U S A. 1992;89(22):10578–82. doi: 10.1073/pnas.89.22.10578.