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Correlation of Expression of CHI3L1 and Nogo-A and their Role in Angiogenesis in Invasive Ductal Breast Carcinoma

AGNIESZKA RUSAK[1], KAROLINA JABLONSKA[1], ALEKSANDRA PIOTROWSKA[1], JEDRZEJ GRZEGRZOLKA[1], ANDRZEJ WOJNAR[2] and PIOTR DZIEGIEL[1,3]

[1]*Division of Histology and Embryology, Department of Human Morphology and Embryology, Wroclaw Medical University, Wroclaw, Poland;*
[2]*Department of Pathology, Lower Silesian Oncology Center, Wroclaw, Poland;*
[3]*Department of Physiotherapy, University School of Physical Education, Wroclaw, Poland*

Abstract. *Background/Aim: Chitinase 3 like 1 (CHI3L1) is a secretion glycoprotein. Elevated levels of this protein are observed in cancer diseases. The biological role of CHI3L1 is not yet fully known, but the connection between CHI3L1 and angiogenesis has been shown. Recent reports also describe the association of Nogo isoforms and Nogo-B receptor (NgBR) with a proliferative potential, cancer cell invasiveness, and angiogenesis. The aim of this study was to evaluate the levels of CHI3L1, Nogo-A, Nogo-A/B, and NgBR and correlate them with clinical-pathological data, to study their role in angiogenesis in invasive ductal breast carcinoma (IDC). Materials and Methods: A total of 77 IDC cases were used in the study. Immunohistochemistry was used to determine the level of expression of CHI3L1, Nogo-A, Nogo-A/B, NgBR and vascular endothelial growth factors (VEGFA, VEGFC and VEGFD). The obtained results were subjected to statistical analysis including clinical-pathological data. Results: A statistically significant positive correlation of CHI3L1 and Nogo-A expression ($r=0.474$, $p>0.0001$) and a positive correlation of Nogo-A and VEGFC expression ($r=0.280$, $p=0.013$) were found. Conclusion: CHI3L1 and Nogo-A are important in angiogenesis in IDC.*

Chitinase 3 like 1 (CHI3L1, YKL-40, HCgp-39, gp-38k) is a 40-kDa glycoprotein composed of 383 amino acids (1). This protein shows a significant homology to the family of chitinase-like proteins (CLPs), but it does not have an enzymatic activity (2). Human *CHI3L1* gene is located at the chromosomal region 1q32.1 and is 8 kbp in size, including 10 exons (2). The function and mechanism of action of CHI3L1 have not yet been fully determined. CHI3L1 regulates cell proliferation (fibroblasts, chondrocytes) and differentiation (macrophages), inflammatory processes, and extracellular matrix reorganization, and also prevents apoptosis (2-4). CHI3L1 involvement in chronic inflammation of varied etiology is commonly described, including autoimmune inflammatory diseases, such as multiple sclerosis (MS) (5-8).

In cancer patients, the concentration of CHI3L1 is elevated and depends, *inter alia*, on the type and stage of cancer, as well as on the localization of metastases (9). CHI3L1 expression was found in cancer cells and tumor-associated macrophages (TAM) (10, 11). An increased serum level of CHI3L1 is considered a negative prognostic factor in breast, lung and ovary carcinoma, as well as in melanoma (5-7). The role of CHI3L1 as a biomarker *e.g.* in breast cancer, is still unclear (12-15). The involvement of CHI3L1 in angiogenesis was indicated by studies in human breast cancer, colon cancer, and glioma (11, 15-17).

Nogo isoforms belong to the family of reticulon proteins (RTN) associated with membranes of the endoplasmic reticulum. There are three main isoforms of Nogo in the fourth group of reticulons (RTN4): Nogo-A (RTN4A, 200 kDa), Nogo-B (RTN4B, 55 kDa) and Nogo-C (RTN4C, 25 kDa) (18-22). These proteins are expressed *e.g.* in the nervous system (Nogo-A, Nogo-B), macrophages (Nogo-A, Nogo-B), skeletal muscle cells (Nogo-A, Nogo-C), as well as in vascular endothelial cells and smooth muscle cells of the blood vessel wall (Nogo-B) (19-22). Reticulons may be involved in processes that include inhibition of neurite growth and axonal regeneration (Nogo-A), promotion of endothelial cell migration and inhibition of migration of smooth muscle cells of the blood vessel wall (Nogo-B), recruitment of leukocytes into the site of chronic or acute

Correspondence to: Agnieszka Rusak, Chalubinskiego 6a, 50-368 Wroclaw, Poland. Tel: +48 717841354(55), Fax: +48 717840082, e-mail: rusakagn@gmail.com

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inflammation, promotion of epithelial–mesenchymal transition (EMT) (NgBR), as well as promotion (Nogo-B, Nogo-C) or inhibition (Nogo-A) of apoptosis (19, 21, 23-28). Nogo-B was shown to mediate chemotaxis and morphogenesis of endothelial cells by binding to the specific NgBR (29). Nogo proteins, especially Nogo-A, are involved in the pathogenesis of neurodegenerative diseases: multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (19, 28, 30-33). The results of studies on the expression and prognostic value of isoforms of Nogo and NgBR in cancers, such as melanoma, gastric, lung or breast cancer, are inconclusive (18, 34-38). Angiogenesis is believed to be one of the processes that involve Nogo proteins (29, 39).

MAPK and PI3K/Akt are common intracellular pathways involving both CHI3L1 and Nogo. Importantly, it is possible that these two pathways are mutually intersecting (16, 40-43). Both of the aforementioned signaling pathways have a crucial role in carcinogenesis, promote cancer cell proliferation and migration, inhibit apoptosis and stimulate angiogenesis (44-47).

Previous *in vitro* studies showed that CHI3L1 is involved in angiogenesis by activating MAPK/ERK and PI3K/Akt pathways in human vessel microvascular endothelial cells, endothelial progenitor cells, as well as in human U87 glioma cells (11, 16, 40, 48, 49). In endothelial cells, CHI3L1 promotes bond formation between syndecan-1 (SDC1) and integrin $\alpha_v\beta_3$, which leads, through FAK^{861} (focal adhesion kinase), to the activation of angiogenesis-promoting MAPK/ERK1/2 pathway (48). In glioma cells, on the other hand, CHI3L1 promotes formation of bonds between syndecan-1 and integrin $\alpha v\beta 5$, and through FAK^{397} it activates the MAPK/ERK1/2 pathway that increases VEGF expression and also promotes angiogenesis (40). Moreover, FAK^{397} activates the PI3K/Akt pathway that protects glioma cells from apoptosis. In glioma cells, PI3/Akt activation by CHI3L1 can presumably take place also by CHI3L1 binding with the interleukin receptor IL-13Rα2 (40).

The importance of Nogo-A as an activator of MAPK/ERK1/2 and PI3K/Akt pathways was demonstrated in an *in vivo* study on neuronal conductivity and regeneration in mice (41, 42). As shown in this study, receptor binding by Nogo-A leads to the activation of RhoA GTPase, PTEN phosphorylation, PI3K/Akt and ERK1/2 activation and, consequently, promotion of neuronal survival (42).

Based on the above literature data, we investigated the potential correlation of CHI3L1 expression with Nogo and NgBR, as well as their involvement in angiogenesis in invasive ductal breast carcinoma (IDC).

Materials and Methods

Patients and tumors. The study was performed on IDC paraffin sections (n=77) taken from patients diagnosed and operated in the Lower Silesian Oncology Center in Wroclaw between 1999-2002. The control materials were non-malignant breast tissue lesions (NBTLs) (n=22). Table I shows selected clinical-pathological characteristics of patients. All studies were conducted with the consent of Ethics Committee of Wroclaw Medical University (consents: KB-616/2014 and KB-735/2017).

Immunohistochemistry (IHC). Collected IDC fragments were fixed in 4% buffered formalin and embedded in paraffin. IHC reactions were performed using Dako Autostainer Link48 (Dako, Glostrup, Denmark) on 4 μm-thick paraffin sections. Deparaffinization, hydratation and epitope unveiling (97˚C, 20min) were conducted using PT- Link apparatus (Dako) in EnVision™ FLEX Target Retrieval Solution High pH (9.0) (Dako) for antibodies against CHI3L1, Nogo-A, NgBR, VEGFA, VEGFD, estrogen receptors (ERs), progesterone receptors (PRs), epidermal growth factor receptor 2 (HER-2) and in EnVision™ FLEX Target Retrival Solution Low pH (6.0) (Dako) for antibodies against Nogo-A/B, VEGFC and Ki-67. Endogenous peroxidase was blocked using EnVision™ FLEX Peroxidase-Blocking Reagent (Dako) (5 min). LSAB+ (Dako) visualization system was used for IHC reaction with goat polyclonal CHI3L1 (1:100, 20 min, RT); R&D Systems, Minneapolis, MN, USA), Nogo-A (1:800, 20 min, RT; Santa Cruz Biotechnology, Dallas, TX, USA), Nogo-A/B (1:3200, 20 min, RT; Santa Cruz), VEGFC and VEGFD (1:100, 18h, 4˚C; ReliaTech GmbH, Braunschweig, Germany) antibody. Using EnVision™ FLEX (Dako) system, IHC reactions were performed with VEGFA (1:50, 18 h, 4˚C; Dako), NgBR (1:100, 20 min, RT; Imgenex, San Diego, CA, USA), Ki-67 (clone MIB-1, ready-to-use (RTU), 20 min, RT; Dako), ER (clone ID5, RTU, 20 min, RT; Dako) and PR (clone PR 626, RTU, 20 min, RT; Dako) antibody. Sections were counterstained with hematoxylin (EnVision™ FLEX Hematoxylin, Dako). HER2 was detected with HercepTest™ kit (Dako). Visualization systems were used according to manufacturer's recommendations.

Analysis of IHC reactions. Immunohistochemical reactions were analyzed using a light microscope (BX41, Olympus, Tokyo, Japan). Photos were taken with a Panoramic MIDI scanner (3DHISTECH, Budapest, Hungary). Intensities of IHC reactions for CHI3L1, Nogo-A, Nogo-A/B, NgBR, VEGFA, VEGFC, and VEGFD were evaluated with semi-quantitative, 12-points score (IRS) by Remmele and Stegner (50), (Table II). The evaluation of IHC reactions with anti-Nogo-A and anti-Nogo-A/B antibodies was taken into account to estimate the expression of Nogo-B. Expression of ER, PR and HER-2 were evaluated using a 0-3 score scale: 0 (0% of positive cells), 1 (1-10% of positive cells), 2 (11-50% of positive cells), 3 (51-100% of positive cells). For ER and PR, the reaction was positive for ≥1% of positive cells (as indicated by the score of 1 or higher) (51). For the evaluation of HER2 expression, the reaction was positive if >10% of cancer cells showed strong membrane reaction (3 points) (52). Nuclear expression of Ki-67 was estimated with the use of 0-4 score scale. The expression of Ki-67 was evaluated as low when ≤25% (the score of 1-2 according to the scale) of cells were positive, and as high when >25% (the score of 3-4 according to the scale) of cells were positive (53). Median was used as a cut-off point for evaluations with the IRS scale. Cases with IRS score 0-3 were estimated as low CHI3L1 expression, whereas IRS scale >3 was indicative of high CHI3L1 expression. In case of VEGFA, VEGFC, and VEGFD, IRS score 0-4 was indicative of low expression, and IRS score 6-12 was indicative of high expression of those proteins. For Nogo-A, no reaction (IRS score 0) vs. positive reaction (IRS score 1-12) was used as a cut-off point (Table III).

Table I. *Analysis of expression of CHI3L1 and Nogo-A (IRS) according to clinical-pathological data; Kruskal–Wallis test, Mann–Whitney U test; medians were used as cut-off points, statistically significant p-values were <0.05.*

			CHI3L1 N (%)					Nogo-A N (%)				
Parameters	N	%	IRS (0-3)	%	IRS (4-12)	%	*p*-Value	IRS (0)	%	IRS (1-12)	%	*p*-Value
Age												
>50	51	66.2	32	62.7	19	37.3		30	58.8	21	41.2	
≤50	26	33.8	20	76.9	6	23.1		14	53.8	12	46.2	
Total	77	100.0	52	67.5	25	32.5	0.965	44	57.1	33	42.9	0.624
Menopausal status												
Pre-menopausal	27	35.1	20	74.1	7	25.9		15	55.6	12	44.4	
Post-menopausal	50	64.9	32	64.0	18	36.0	0.987	29	58.0	21	42.0	0.934
Tumor grade												
G1	7	9.1	5	71.4	2	28.6		3	42.9	4	57.1	
G2	40	51.9	26	65.0	14	35.0		23	57.5	17	42.5	
G3	30	39.0	21	70.0	9	30.0	0.993	18	60.0	12	40.0	0.642
Stage												
I	26	33.8	20	76.9	6	23.1		16	61.5	10	38.5	
II	39	50.6	22	56.4	17	43.6		21	53.8	18	46.2	
III	11	14.3	9	81.8	2	18.2		7	63.6	4	36.4	
IV	1	1.3	1	100.0	0	0.0	0.461	0	0.0	1	100.0	0.933
Tumor size												
pT1	44	57.1	30	68.2	14	31.8		25	56.8	19	43.2	
pT2	26	33.8	17	65.4	9	34.6		17	65.4	9	34.6	
pT3	4	5.2	3	75.0	1	25.0		1	25.0	3	75.0	
pT4	3	3.9	2	66.7	1	33.3	0.804	1	33.3	2	66.7	0.208
Lymph node												
pN0	43	55.8	30	69.8	13	30.2		26	60.5	17	39.5	
pN1	24	31.2	14	58.3	10	41.7		13	54.2	11	45.8	
pN2	6	7.8	5	83.3	1	16.7		5	83.3	1	16.7	
pN3	2	2.6	1	50.0	1	50.0	0.204	0	0.0	2	100.0	0.158
Unknown	2	2.6	2	100.0	0	0.0		0	0.0	2	100.0	
Hormonal status												
ER status												
Negative	18	23.4	10	55.6	8	44.4		11	61.1	7	38.9	
Positive	59	76.6	42	71.2	17	28.8	0.069	33	56.0	26	44.0	0.458
PR status												
Negative	27	35.1	15	55.6	12	44.4		17	63.0	10	37.0	
Positive	50	64.9	37	74.0	13	26.0	0.085	27	54.0	23	46.0	0.299
HER-2												
Negative	63	81.8	42	66.7	21	33.3		35	55.6	28	44.4	
Positive	14	18.2	10	71.4	4	28.6	0.616	9	64.3	5	35.7	0.164
TN	13	16.9	8	61.5	5	38.5		9	69.2	4	30.8	
Others	64	83.1	44	68.8	20	31.3	0.416	35	54.7	29	45.3	0.971
Ki-67												
Low ≤25%	54	70.1	37	68.5	17	31.5		32	59.3	22	40.7	
High >25%	22	28.6	14	63.6	8	36.4	0.834	12	54.6	10	45.4	0.668
Unknown	1	1.3	1	100.0	0	0.0		0	0.0	1	100.0	

CHI3L1: Chitinase 3 like 1; Nogo-A: neurite outgrowth inhibitor type A; ER: estrogen receptor; PR: progesterone receptor; HER-2: human epidermal growth factor receptor 2; TN: triple negative (no expression of ER, PR and HER-2 receptor).

Statistical analysis. Normal distribution analysis was performed with the Shapiro–Wilk test. The levels of expression of the studied markers were statistically analyzed with the use of Mann–Whitney *U*-test and Kruskal–Wallis test (one-way ANOVA on ranks) using post-hoc Dunn's test. Correlation was calculated using Spearman's test. Mantel–Cox test (Log-rank) was used for survival analysis. Statistically significant *p*-values were ≤0.05. Prism 5.0 (GraphPad, La Jolla, CA, USA) and STATISTICA 12.0 (Statsoft, Tulsa, OK, USA) software was used for statistical analysis.

Results

IHC expression of CHI3L1, Nogo, and VEGF. The results of the IHC indicated cytoplasmic localization of CHI3L1, Nogo-A, Nogo-A/B, and NgBR, as well as of selected angiogenesis markers: VEGFA, VEGFC, and VEGFD in IDC cancer cells (Figure 1). Table III shows the intensity of the IHC reactions for the studied antigens, as well as the cut-off points indicated

Table II. *Immunohistochemical reaction intensity (IRS) according to Remmele and Stegner (50). The score results from multiplying immunopositive cell percentage (A) with immunohistochemical reaction intensity (B); (IRS=AxB).*

Points	Percentage of positively stained cells (A)	Intensity of color reaction (B)
0	0%	0 No color reaction
1	≤10%	1 Low color intensity
2	11-50%	2 Moderate color intensity
3	51-80%	3 Intense color
4	>80%	

by the median score. More than 30% of the analyzed IDC cases were characterized by a high level of CHI3L1 expression. Positive IHC reaction for Nogo-A was reported in 33 (42.86%) cases. More than half of the cases (49) showed low NgBR expression. Almost 80% of cases were characterized by low VEGFA expression, whereas 39 (50.6%) and 41 (53.2%) cases had low expression of VEGFC and VEGFD. The analysis of the obtained results showed a statistically significant positive correlation of Nogo-A and CHI3L1 expression (r=0.474, $p>0.0001$) (Figure 2A) and positive correlation of Nogo-A and VEGFC expression (r=0.280, $p=0.013$) (Figure 3B). Additionally, an almost statistically significant correlation of Nogo-A with VEGFA and VEGFD (r=0.211, $p=0.065$; r=0.192, $p=0.094$) was observed (Figure 3A and C). No correlation of CHI3L1 and Nogo-A/B (Figure 2B) and also CHI3L1 and NgBR was observed (Figure 2C).

Analysis with clinical-pathological data. The relationship between the level of expression of CHI3L1, Nogo-A and clinical-pathological patient data are shown in Table I. No differences were observed in the expression of CHI3L1 and Nogo-A depending on tumor grade of malignancy, Ki-67 expression, tumor size, lymph node metastases and distant metastases (pTNM), stage of cancer or status of ER, PR and HER-2 receptors. Also, statistical analysis showed no correlation of Nogo-A expression with the age of patients nor with menopause. The analysis performed did not show a causal link between the expression of CHI3L1 and Nogo-A and patient overall survival (OS) or disease-free survival (DFS) (Table IV).

Discussion

Multiple *in vitro* and *in vivo* studies have suggested the potential involvement of CHI3L1 in angiogenesis associated with carcinogenesis by promoting endothelial cell migration and participating in vascular structure formation (11, 16, 40). The results of *in vitro* studies using human microvessel endothelial cells (HMVEC) and U87 human glioma cell line showed a synergistic CHI3L1 and VEGF activity in vascular structure formation (16, 40). Studies performed with the use of a mouse model for human breast cancer, colon cancer and glioma, as well as with the patient glioma material, highlighted the role of CHI3L1 in promoting angiogenesis (11, 16, 40, 54). Moreover, in one of our recently published research on IDC, a positive correlation was shown between CHI3L1 and angiogenesis markers such as: CD31, CD34, and VEGFD. This also suggested that there is a link between CHI3L1 and angiogenesis in this type of cancer (15).

Previous breast cancer studies performed on tissue specimens indicated a lack of correlation between CHI3L1 expression and clinical-pathological data, such as: the presence of lymph node metastases, tumor size, patient's age, OS and DFS, which was also confirmed by our results (12-15, 55). However, studies on the blood serum of breast cancer patients indicated a correlation between an increased level of CHI3L1 and shorter OS and DFS, which does not exclude the prognostic potential of CHI3L1 in IDC (56-58).

In this work, we showed for the first time, a positive correlation of CHI3L1 with Nogo-A and of Nogo-A with VEGFC, which may suggest a link between Nogo-A and blood vessel formation in breast cancer. This hypothesis is based on the results of studies on the role of CHI3L1 and Nogo-A in the regulation of angiogenesis by MAPK and PI3K/Akt pathways (40-42). These pathways are also activated in VEGF-dependent angiogenesis (59, 60). The published results describe Nogo-A as a negative regulator of angiogenesis in the central nervous system (CNS) (28, 61). It is believed that by activating Rho-A/Rock/myosin II pathway, Nogo-A plays an inhibitory role in angiogenesis, migration, adhesion and budding of endothelial cells (61). So far, Nogo-A expression on a protein level was found in oligodendrocytes, neurons and glial cells, whereas in endothelial cells Nogo-A expression was observed only at the mRNA level (28, 61). In this work, we did not observe any link between the levels of Nogo-A and selected clinical-pathological factors, nor with OS and DFS in IDC. Up until now, there is no literature where similar correlations are described.

In our studies, we did not observe any statistically significant correlations between CHI3L1 and Nogo-A/B or NgBR. It is noteworthy that thus far clear correlations between these proteins have not been described. It has been shown that Nogo-B is involved in blood vessel remodeling through the promotion of HUVEC endothelial cell migration and in inhibition of human aortic vascular smooth muscle cells (HAVSMC) migration (20). In human endothelial cell HUVEC line and in mouse microvascular endothelial cell MVEC line, Nogo-B was identified at the protein level (20, 28, 61). Drożdż *et al*., have reported that the levels of Nogo-B mRNA and protein expression in the internal membrane of blood vessels from atherosclerotic patients were lower compared to control (62). Zhao *et al*. described a role of Nogo-B and NgBR in blood vessel formation in zebrafish

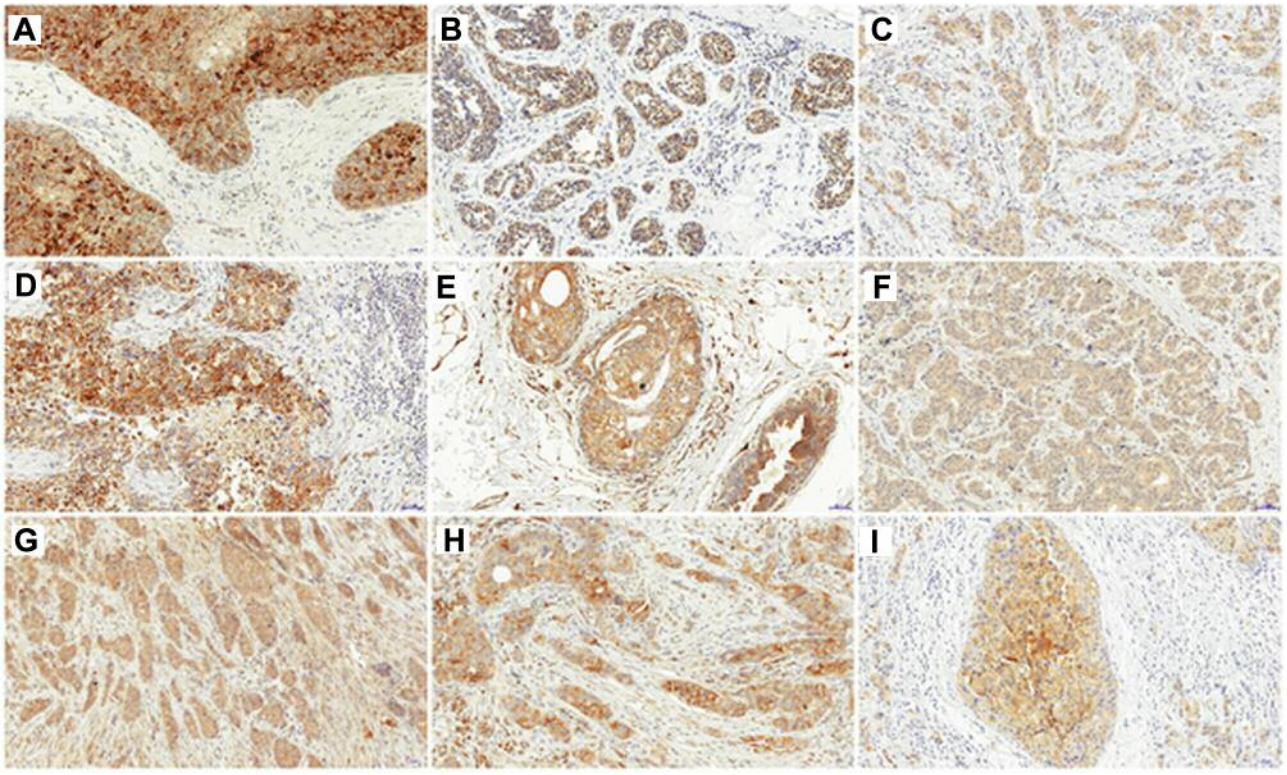

Figure 1. *Immunohistochemical reactions (IHC) performed on paraffin sections of invasive ductal breast carcinoma (IDC). Cytoplasmic localization in cancer cells: chitinase 3 like 1 (CHI3L1) (A - intense, B - medium), neurite outgrowth inhibitor type A (Nogo-A) (D - intense, C - medium), Nogo-A/B (E), receptor for Nogo-B (NgBR) (F), vascular endothelial growth factors: VEGFA (G), VEGFC (H), and VEGFD (I). Magnification 200×.*

Table III. *Intensity of evaluated immunohistochemical reactions. Semi-quantitative immunoreactive score (IRS) scale according to Remmele and Stegner (IRS) was used; medians were used as cut-off points.*

IHC marker	Cut-off (IRS)	Low expression (N)	%	High expression (N)	%
CHI3L1	0-3 *vs*. 4-12	52	67.5	25	32.5
Nogo-A	0 *vs*. 1-12	44	57.1	33	42.9
Nogo-A/B	0-6 *vs*. 8-12	47	61.0	30	39.0
NgBR	0-4 *vs*. 6-12	49	63.6	28	36.4
VEGFA	0-6 *vs*. 8-12	60	77.9	17	22.1
VEGFC	0-4 *vs*. 6-12	39	50.6	38	49.4
VEGFD	0-4 *vs*. 6-12	41	53.2	36	46.8

CHI3L1: Chitinase 3 like 1; Nogo-A: neurite outgrowth inhibitor type A; Nogo-A/B: neurite outgrowth inhibitor type A/B; NgBR: neurite outgrowth inhibitor type B receptor; VEGFA: vessel endothelial growth factor A; VEGFC: vessel endothelial growth factor C; VEGFD: vessel endothelial growth factor D.

embryos (39). Currently, there are no publications on the role of Nogo-B and NgBR in angiogenesis in IDC.

On the other hand, there are many papers emphasizing on the role of Nogo proteins in carcinogenesis. The results of *in vitro* studies on MCF7 and MCF10AT cell lines showed the impact of Nogo-A on the proliferation and migration of breast cancer cells (63). The results of our studies on non-small cell lung cancer (NSCLC) showed high level of Nogo-A/B expression in squamous cell carcinoma in comparison with adenocarcinoma. Also, low levels of Nogo-A/B expression were reported in higher stage NSCLC (18). Low levels of Nogo-A/B expression in melanoma and increased levels of its expression in gastric cancer were described as an unfavorable prognostic factor

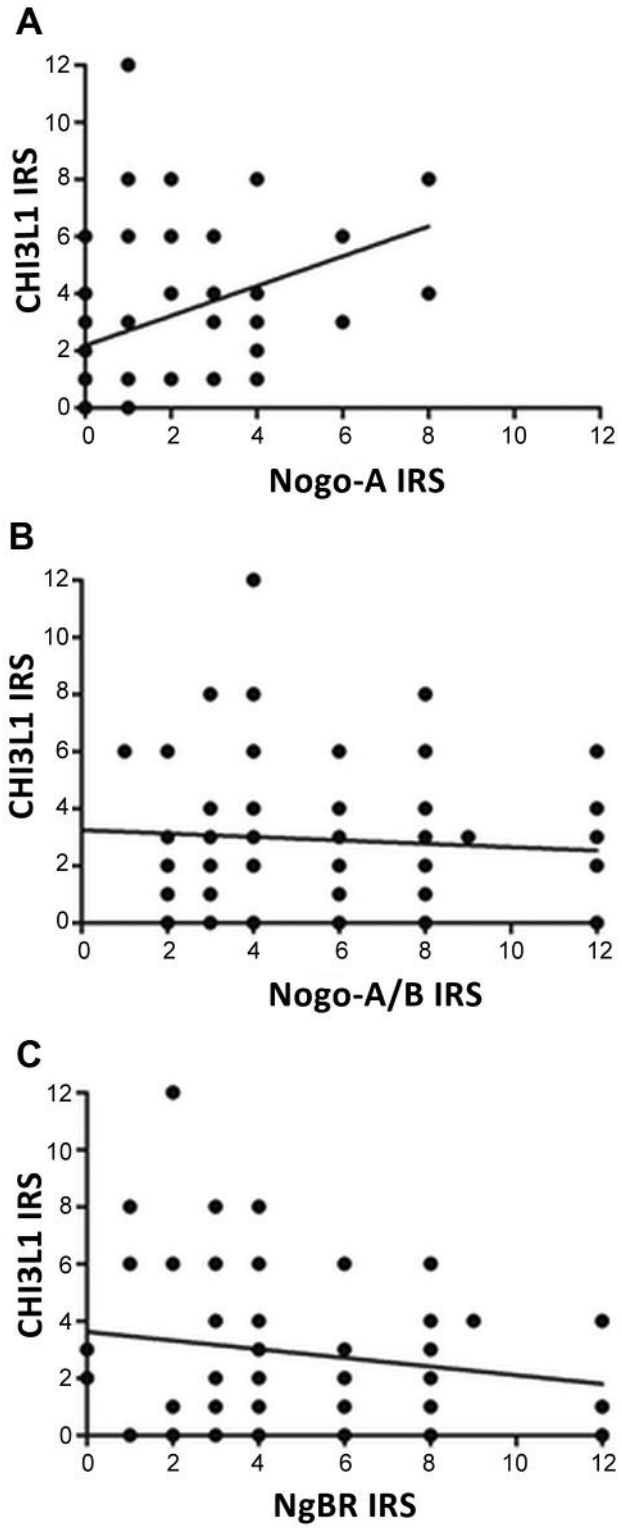

Figure 2. *Correlation (IRS) between CHI3L1 (chitinase 3 like 1), Nogo-A, Nogo-A/B (neurite outgrowth inhibitor) and NgBR (neurite outgrowth inhibitor type B receptor) in invasive ductal carcinoma (IDC). A) CHI3L1 vs. Nogo-A: r=0.474, p<0.0001; B) CHI3L1 vs. Nogo-A/B: r=–0.020, p=0.855; C) CHI3L1 vs. NgBR: r=–0.116, p=0.314; Spearman's correlation test, p<0.05.*

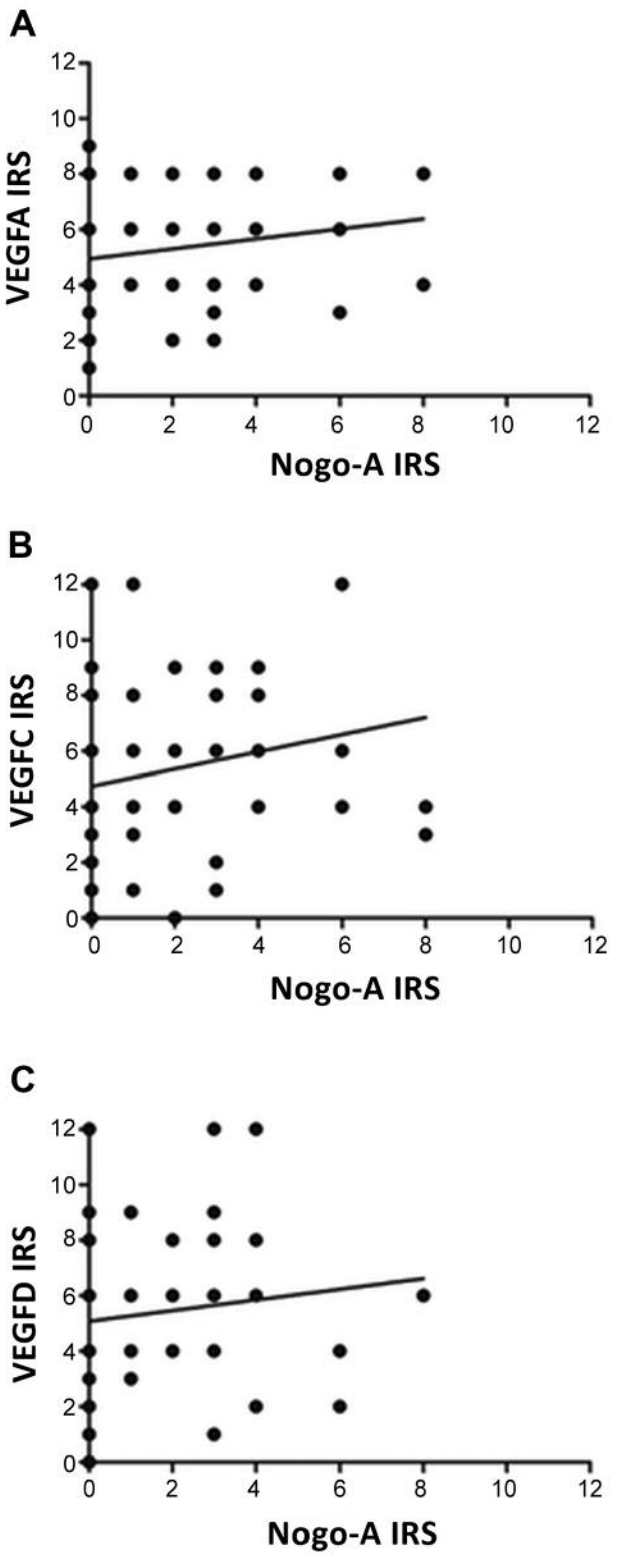

Figure 3. *Correlation of (IHC) Nogo-A with vascular endothelial growth factors (VEGFA, VEGFB, VEGFC) in invasive breast carcinoma. A) Nogo-A vs. VEGFA, r=0.211, p=0.065; B) Nogo-A vs. VEGFC, r=0.280, p=0.013; C) Nogo-A vs. VEGFD, r=0.192, p=0.094; Spearman's correlation test, p<0.05.*

Table IV. *The analysis of overall survival and disease-free survival for 77 patients diagnosed with IDC [Mantel–Cox test (Log-rank)]. Immunohistochemical reactions were evaluated according to Remmele and Stegner score (IRS) (50); medians were used as cut-off points, statistically significant p-values were <0.05.*

IHC marker	N	Cut-off (IRS)	HR	95%CI	*p*-Value
CHI3LI	77	0-3 *vs*. 4-12	1.367	0.665-2.810	OS: 0.396
			0.912	0.409-2.032	DFS: 0.821
Nogo-A	77	0 *vs*. 1-12	0.987	0.494-1.972	OS: 0.970
			0.802	0.369-1.744	DFS: 0.578

CHI3L1: Chitinase 3 like 1; Nogo-A: neurite outgrowth inhibitor type A; OS: overall survival; DFS: disease-free survival; HR: hazard ratio; CI: confidence interval.

(34, 35). In our own research on IDC, we showed a higher level of NgBR in cancer cells in comparison to the control (NBTL), both at mRNA and protein level. Moreover, NgBR expression in IDC was negatively correlated with a degree of histological malignancy and Ki-67, whereas lower levels of NgBR mRNA may be considered an unfavorable prognostic factor for patient overall survival (36). On the other hand, Wang *et al*. showed a higher level of NgBR expression in tumors with higher grade of histological malignancy (II-IV) and positive correlation of NgBR and survivin expression in IDC (37). Also, a higher NgBR expression was observed in ER positive (ER+), as well as in HER2 negative tumors (HER2-) (37). Higher levels of NgBR expression in hepatocellular carcinoma patients were correlated with shorter OS (38).

Our study suggests that CHI3L1 and Nogo-A may be important in angiogenesis in IDC. CHI3L1 and Nogo-A are supposedly involved, inter alia, in inflammatory processes (28, 48, 49). We presume that the pro-angiogenic role of CHI3L1 and Nogo-A may be correlated with an inflammatory process associated with cancer. This hypothesis, however, requires further study to evaluate the role of CHI3L1 and Nogo-A in the regulation of PI3K/Akt and MAPK pathways, which are so important in oncogenesis and angiogenesis (40-42, 45, 64, 65).

Conclusion

A correlation between the expression of CHI3L1 and Nogo-A was found in IDC which indicates a possible involvement of Nogo-A in angiogenesis (positive correlation with VEGFC). These results are a prelude for further study that could precisely determine the function of CHI3L1 and Nogo-A in IDC.

Conflicts of Interest

The Authors declare that they have no competing interests in regard to this study.

Authors' Contributions

Design, data collection: AR, AP, JG, AW. Writing of the manuscript: AR. Revision of the manuscript: KJ, PD. All Authors approved the final manuscript.

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