Research Article

Immunohistochemical Expression of Claudin-4 in Invasive Ductal Carcinoma of the Breast

Dalia M. Abd El-Rehim, Rabab A. Safwat, Al Zahraa I. Khalil, and Reham D. Al-Azhary .
Department of Pathology, Faculty of Medicine, Minia University, Egypt.

Abstract
Background: Breast carcinoma is a major public health problem. Claudins are important tight junction proteins, they came into focus for their suggested role in carcinogenesis and possible role in cancer therapy. To date, few studies have addressed the role of claudins in BC. Claudin-4 has been found to be overexpressed in a wide variety of cancers including breast cancer, its role in BC progression and spread constitutes an active research focus. **Methods:** Immunohistochemical staining of claudin-4 was conducted on 50 tissue specimens of invasive ductal carcinoma of the breast (IDC-NST) by using the avidin biotin-peroxidase complex method with diaminobenizidine (DAB) chromagen detection system. **Results:** High claudin-4 expression was detected in 52% of cases. Claudin-4 expression showed statistically significant association with tumor laterality, tumor grade, stage, advanced nodal status, LNR, NPI, Ki67 PI, ER& PR status and triple negative phenotype (*p.*= 0.008, 0.027, 0.016, 0.043, 0.006, 0.006, <0.001, 0.014, 0.019 and 0.047 respectively). On comparing claudin-4 expression in 12 pairs of primary IDC-NST and their corresponding LN metastasis, a concordance rate of 75% was found between primary tumors and their metastatic LNs where 9/12 cases maintained the same expression score. **Conclusions:** Claudin-4 expression is suggested to be a molecular protein with an oncogenic function and biological value. Being involved in IDC-NST progression and its association with many poor prognostic factors of IDC-NST, the important role in progression and spread in invasive ductal breast carcinoma is highlighted.
Key words: Claudin-4, Breast Carcinoma, immunohistochemistry.

Introduction
Breast carcinoma (BC) is the second most common cancer worldwide after lung carcinoma and a leading cause of mortality and morbidity, representing a major public health problem. In Egypt, it is the second most common cancer after liver carcinoma representing 17.9% of all cancers (Ferlay 2019). A gradual decline in the incidence of BC is documented in developed countries especially over last two decades, while in developing countries the incidence continues to rise (Fernando 2018). It is a heterogeneous disease that harbors various genetic alterations allowing it to be classified into distinct molecular subtypes that respond differently to therapy and are associated with various clinical outcomes (Zardavas 2015).

Claudins (CLDNs) are essential tetraspan transmembrane proteins of tight junctions (TJs), which form the paracellular permselective barriers. There are 27 known members of the CLDN family, which are expressed in a tissue-specific manner (Mineta, 2011).The main functions of claudins are: (1) Fence function, responsible for maintaining polarity by differentiating apical and basolateral cell domains; (2) Signaling molecule, involved in cell growth, survival, proliferation, and differentiation; (3) Barrier function, this gate function separates compartments with fluids to avoid intermixing (Gowrikumar 2019).

Over-expression or down-regulation of claudins is frequently observed in epithelial-derived cancers. However, molecular mechanisms by which claudins affect tumorigenesis remain largely unknown (Ding 2013). Claudins came into focus for their suggested role in carcinogenesis and possible role in cancer therapy (Katayama 2017). Complex and diverse expression patterns of CLDNs are associated with their diverse functions in tumorigenesis or metastasis (Kwon, 2013).

Claudin-4 has been found to be overexpressed in a wide variety of cancers such as gastric cancer, pancreatic cancer (Nichols 2004; Cunningham 2006), prostate cancer, ovarian cancer, endometrial cancer, and breast cancer (Toke´s 2005; Sheehan 2007; Konecny 2008). Claudin-4 is expressed at higher levels in most tumor cells compared to the normal epithelium of origin. Additionally, it is often found outside of tight junction structures, mainly along basolateral membranes in normal epithelium. These observations suggest that claudin-4 may have functions outside of its traditional barrier forming role in tight junctions and may even participate in activities attributed to the more mesenchymal-like behavior of tumor cells (Hicks 2016).

Previous literature on claudin-4 role in BC has been contradictory: one study found that claudin-4 expression was lost in Grade 1 invasive breast tumors, with increased expr-ession detected in Grade 2 and 3 tumors; others have found that claudin-4 is overexpressed in primary breast carcinomas compared to normal mammary epithelium (Kominsky 2004; Tokes 2005). Another study has described the association of increased expression of claudin-4 with high tumor grade, ER-negative tumors, and poor prognosis (Lanigan 2009).

Recently, a claudin-low phenotype of BC was described as a new subtype by gene microarray. It is typically triple negative by immunehisto-chemistry (IHC) and accounts for 25–39% of triple negative breast carcinoma (TNBC). Defined by low mRNA expression of CLDN3, CLDN4, and CLDN7, this subtype was reported to be a frequent phenomenon in metaplastic and basal-like BCs and has been shown to have a poor prognosis similar to that of basal-like BCs. However, the expression profiles of CLDNs in TNBC have not yet been well analyzed (Ma et al.2014). A few published studies focus on the expression patterns of CLDNs in TNBC. Therefore, their knowledge of the role of CLDNs in the progression of TNBC is insufficient (Katayama 2017).

Material and Methods

1. Tissue specimens

The present study comprised 50 selected cases of invasive ductal carcinomas of the breast. The histopathological grading was done according to the Nottingham Histologic Score System (the Elston-Ellis modification of Scarff-Bloom-Richardson grading system) (Elston and Ellis, 1991). Tumors were staged according to American joint committee on cancer (AJCC) TNM staging (AJCC, 2017). Nottingham prognostic index (NPI) was calculated using the following formula: NPI = tumor size (cm) X (0.2) + lymph node stage (1, 2, or 3) + histologic grade (1, 2, or 3) (Serrero 2016). The Lymph node ratio (LNR) was calculated as the total number of positive lymph nodes divided by the total number of lymph nodes found and examined (LN+/total LN received) (Jayasinghe 2015).

2. Immunohistochemistry

Primary antibody against claudin-4: Claudin-4 mouse antibody (7ml, ready to use catalogue number: MC0209RTU7, Clone A12, Medaysis company). For negative control the primary antibody was replaced with PBS. Positive control was normal human appendix.

3. Scoring of Immunostaining

Claudin-4 expression was mainly membranous. Immunoreactivity was assessed in at least 5 high-power fields and based on combined score of the intensity (0, no stain; 1, weak; 2, moderate; and 3, strong) and the percentage of stained tumor cells (0, <5%; 1, 5%-25%; 2, 26%-50%; and 3, >51%). The final score was calculated by multiplying both scores to give an overall score of 0 to 9, of which 0 was consi-dered negative; 1 to 2 was considered weak; 3 to 6, moderate; and 7 to 9, strong staining. To simplify statistical analysis, negative and weak expression scores were considered as low claudin-4 expression score, whereas moderate and strong expression scores were considered as high claudin-4 expression score (Sheehan 2007).

Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS software version 20).

Results

Clinicopathological Features

Data regarding different clinical and histo-pathological features for invasive ductal carcinoma patients are summarized in **Table (1).**

Table (1): Clinicopathological features for patients with IDC- NST (n=50)

Clinicopathological features		No. (%)
Age		
≤50		24 (48%)
>50		26 (52%)
Laterality		
Right breast		18 (36%)
Left breast		32 (64%)
Tumor grade		
	II	29 (58%)
	III	21 (42%)
Tumor size	**T1**	6 (12%)
	T2	35 (70%)
	T3	9 (18%)
Insitu component		
Absent		26 (52%)
Present		24 (48%)
Nodal status	**N0**	14 (28%)
	N1	18 (36%)
	N2	10 (20%)
	N3	8 (16%)
Lymph node ratio		
Low risk		23 (46%)
Intermediate risk		18 (36%)
High risk		9 (18%)
AJCC stage	**I**	1 (2%)
	II	31 (62%)
	III	18 (36%)
Nottingham prognostic index (NPI)		
Good		2 (4%)
Moderate		26 (52%)
Poor		22 (44%)
Lymphocytic Infiltration		
Absent		20 (40%)
Present		30 (60%)
Necrosis		
Absent		32 (64%)
Present		18 (36%)
Lymphovascular invasion		
Absent		42 (84%)
Present		8 (16%)
Ki67		
<14%		4 (8%)
>14%		46 (92%)
Estrogen receptor		
Negative		24 (48)
Positive		26 (52)
Progesterone receptor		
Negative		24 (48)
Positive		26 (52)
HER2 receptor		
Negative		42 (84%)
Positive		8 (16%)
Molecular subtype		
Luminal A		2 (4%)
Luminal B		24 (48%)
HER 2 +		4 (8%)
TNBC		20 (40%)

2. Immunohistochemical expression of claudin-4 and its association with patients' clinicopathological features

Table (2): Association between claudin-4 expression and clinicopathological features for patients with IDC- NST (n=50)

Clinicopathological features	Total 50 (100%)	Claudin-4 expression		*P* value
		low expression 21 (42%)	High expression 29 (58%)	
Age				
≤50	24 (48%)	10 (41.7)	14 (58.3)	0.963
>50	26 (52%)	11(42.3)	15 (57.7)	
Laterality				
Right breast	18 (36%)	12 (66.7)	6 (33.3)	0.008*
Left breast	32 (64%)	9 (28.1)	23 (71.9)	
Tumor grade				
II	29 (58%)	16 (55.2)	13 (44.8)	0.027*
III	21 (42%)	5 (23.8)	16 (76.2)	
Tumor size				
T1	6 (12%)	3 (50)	3 (50)	0.406
T2	35 (70%)	16 (45.7)	19 (54.3)	
T3	9 (18%)	2 (22.2)	7 (77.8)	
Insitu component				
Absent	26 (52%)	12 (46.2)	14 (53.8)	0.536
Present	24 (48%)	9 (37.5)	15 (62.5)	
Nodal status				
N0	14 (28%)	9 (64.3)	5 (35.7)	
N1	18 (36%)	9 (50)	9 (50)	0.043*
N2	10 (20%)	2 (20)	8 (80)	
N3	8 (16%)	1 (12.5)	7 (87.5)	
Lymph node ratio				
Low risk	23 (46%)	15 (65.2)	8 (34.8)	0.006*
Intermediate risk	18 (36%)	5 (27.8)	13 (72.2)	
High risk	9 (18%)	1 (11.1)	8 (88.9)	
AJCC stage				
I	1 (2%)	1 (100)	0 (0)	0.016*
II	31 (62%)	17 (54.8)	14 (45.2)	
III	18 (36%)	3 (16.7)	15 (83.3)	
Nottingham prognostic index				
Good	2 (4%)	2 (100)	0 (0)	<0.001*
Moderate	26 (52%)	17 (65.4)	9 (34.6)	
Poor	22 (44%)	2 (9.1)	20 (90.9)	
Lymphocytic Infiltration				
Absent	20 (40%)	10 (50)	10 (50)	0.349
Present	30 (60%)	11 (36.7)	19 (63.3)	
Necrosis				
Absent	32 (64%)	16 (50)	16 (50)	0.126
Present	18 (36%)	5 (27.8)	13 (72.2)	
Lymphovascular invasion				
Absent	42 (84%)	18 (42.9)	24 (57.1)	0.778
Present	8 (16%)	3 (37.5)	5 (62.5)	
Ki67				
<14%	4 (8%)	4 (100)	0 (0)	0.014*

>14%	46 (92%)	17 (37)	29 (63)	
Estrogen receptor				
Negative	24(48)	6 (25)	18 (75)	0.019*
Positive	26 (52)	15 (57.7)	11 (42.3)	
Progesterone receptor				
Negative	24 (48) 26	6 (25)	18 (75)	0.019*
Positive	(52)	15 (57.7)	11 (42.3)	
Her2 receptor				
Negative	42 (84%)	17 (40.5)	25 (59.5)	0.617
Positive	8 (16%)	4 (50)	4 (50)	
Molecular subtype				
Luminal A	2 (4%)	2 (100)	0 (0)	
Luminal B	24 (48%) 4	13 (54.2)	11 (45.2)	0.070
HER 2 +	(8%)	1 (25)	3 (75)	
TNBC	20 (40%)	5 (25)	15 (75)	

Test of significance by Chi-square and Fischer exact tests, *p.*<0.05 is considered significant, significant (*)**.**

In the current study, a statistically significant association was detected between claudin-4 expression and tumor laterality, tumor grade, stage, advanced nodal status, LNR, NPI, Ki67 PI, ER& PR status and triple negative phenotype (*p.*= 0.008, 0.027, 0.016, 0.043, 0.006, 0.006, <0.001, 0.014, 0.019 and 0.047 respectively). Among lymph node positive cases claudin-4 expression was evaluated in 12 pairs of the primary tumors and their corresponding lymph node metastases. A concordance rate of 75% in claudin-4 expression was found.

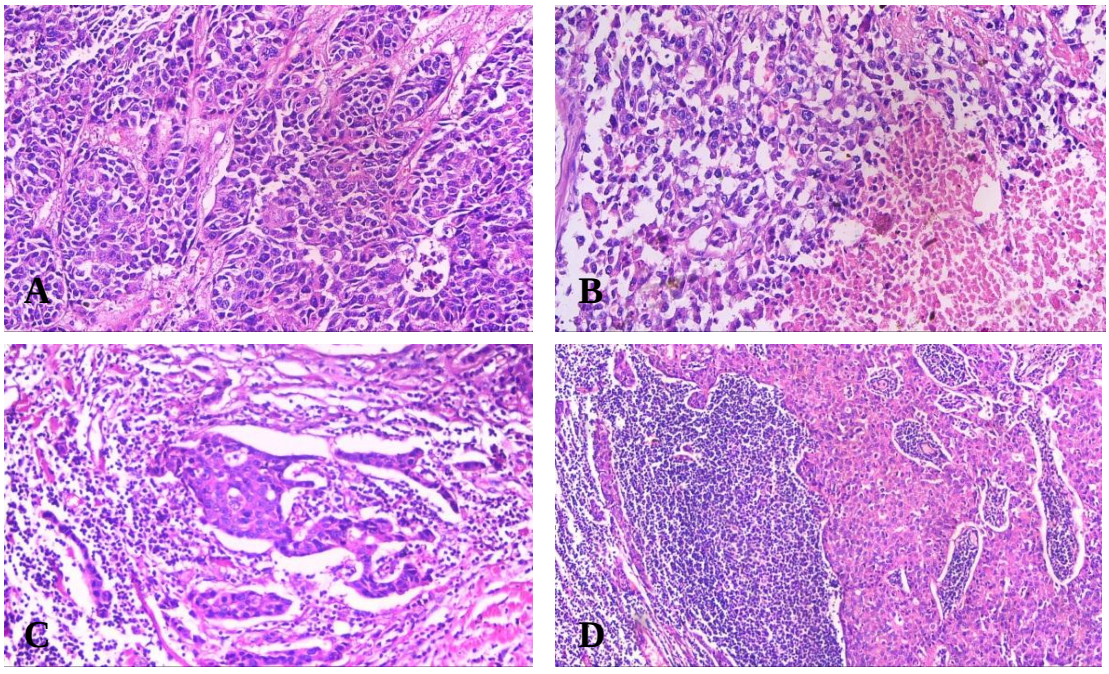

Figure (1): Microphotographs showing histopathological changes observed in breast cancer tissues. A: grade III IDC-NST. B: tumor necrosis in IDC-NST. C: Lymphocytic infiltration in IDC-NST. D: Lymph node metastasis in IDC-NST (H&Ex200).

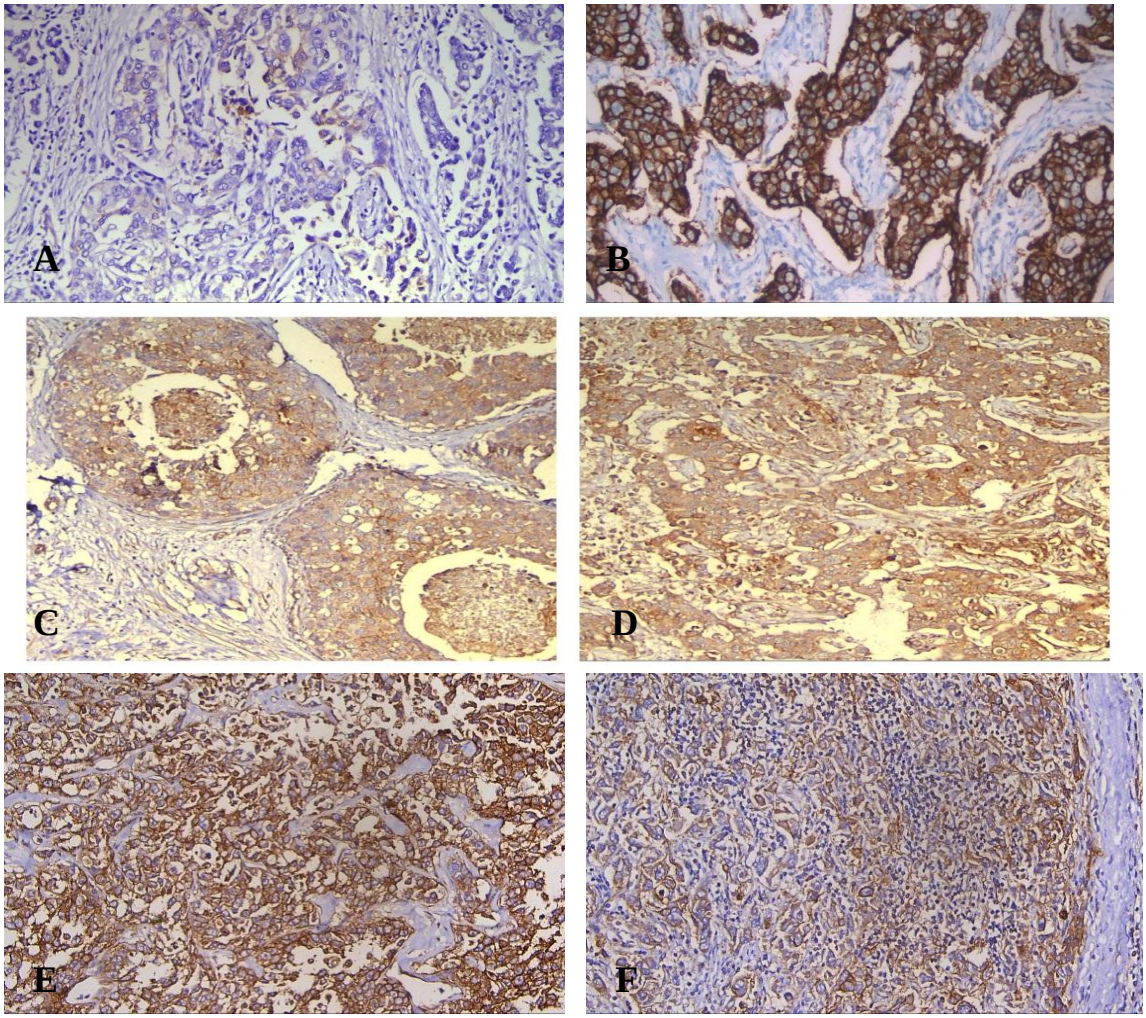

Figure (2): Immunohistochemical expression of claudin-4. A: -ve/low expression in grade III IDC-NST. B: high expression in grade III IDC-NST. C: and D: in situ component and invasive component of IDC-NST**.** E and F: in both primary tumor and metastatic lymph node. (Immunohistochemistry, DAP chromogen and Hematoxylin counterstain x200 in A&B and x 100 in B,C,D &F).

Discussion

The role of claudins in cancer has attracted the attention of many recent researches and few studies have addressed the role of claudin-4 in BC. The study on claudin-4 expression in invasive ductal carcinoma of no special type (IDC-NST) remains controversial and conflicting results were reported regarding its role in BC (Logullo 2018). Therefore, the present study was conducted to evaluate the expression of claudin-4 and its association with clinicpathologic variables in IDC-NST.

Additionally, the change in the expression pattern of claudin-4 in the primary tumor and their corresponding lymph node metastases was evaluated. Based on molecular classification, the present study identified luminal A, luminal B, HER2 enriched and TNBC molecular subtypes in 4%, 48%, 8% and 40% of BC, respectively. These proportions reflected great difference compared to other studies (Adly 2010), particularly luminal A and triple negative subtypes. This could be related to bias in case selection in the current study. A previous large scale study reported that BC is more likely to be diagnosed as luminal B (either HER2 -ve or HER2+ve) (Cheng 2018). The results of this study identified high claudin-4 expression in 58% of IDC-NST cases. Similarly, earlier studies have reported claudin-4 over expression in BC with over expression rates in 58.4%, 41% and 62.6% of BC cases, respectively (Lanigan 2009; Lu 2013; Logullo 2018).

The immunoreactivity for claudin-4 in this study was mainly membranous with some cases showed associated cytoplasmic expression. This was consistent with Lanigan et al. (2009) who found that staining was predominantly membranous, with some claudin-4 positive samples displaying a low level of cytoplasmic staining and Lu et al. (2013) who also described that claudin-4 staining varied from a punctate to complete circumferential membranous staining.

Claudin-4 expression was significantly associated with high tumor grade. This was in accordance with previous studies that reported significant association between high claudin-4 expression and tumor grade (Kulka 2009; Lanigan 2009; Abd-Elazeem and Abd-Elazeem, 2015; Ahmed 2017; and Logullo 2018). The claudin-4 expression among different grades of differentiation requires further investigations in a larger cohort of BC cases.

A statistically significant association was found between claudin-4 expression and advanced lymph node status. This was in agreement with Logullo (2018) who also described a positive association between high claudin-4 expression and advanced lymph node involvement. However, Lanigan (2009) reported that claudin-4 expression was found to be independent of lymph node metastasis.

Furthermore, a statistically significant positive association was found between high claudin-4 expression and advanced stage, where 83.3% of the cases with stage III tumors showed high claudin 4 expression. This was in agreement with Ahmed (2017) who detected high claudin 4 expression in 80% of stage III tumors cases. However, another study reported non-significant association between claudin-4 expression and tumor stage (Katayama, 2017) .

In support of our findings, Ma and his colleagues reported significant increase in migration of claudin-4 overexpressing breast cancer cell lines while claudin-4 knock down significantly reduced cell migration in MCF-7 breast cancer cell line (Ma, 2015). Furthermore, Hicks and his coworkers reported that loss of claudin-4 expression in ovarian cancer cells, expressing high level of claudin-4, reduced cell migration. Their explanation that claudin-4 interacts with protein molecules at the tumor cell surface through extracellular loop interactions and alters intracellular signaling pathways to promote tumor cell survival and migration (Hicks, 2016).

A high proliferative potential is one of the most important characteristics of cancer. In this context the present study investigated the possible association of claudin-4 expression and Ki67 as a reliable proliferative marker in cancer. There was a statistically significant association between high claudin-4 expression score and high Ki67 PIs. This finding was in agreement with previous studies (Abd-Elazeem and Abd-Elazeem, 2015; Blanchard 2009; Katayama 2017). A previous experimental study on MCF-7 breast cancer cell line reported the role of claudin-4 in enhancement of BC cell proliferation through interaction with regulatory proteins, Rab3b and Rab13 (members of RAS oncogene family) and transcription factors such as Zo-1 associated nucleic acid binding protein (ZONAB) involved in cell proliferation (Ma et al., 2015).

A significant positive association was found between high claudin-4 expression and hormone receptor status in the current study. This was in agreement with Logullo et al., (2018) who reported that 70% of ER negative cases and 67.5 % of PR negative cases showed high expression for claudin-4. Also, the significant association between claudin-4 expression and ER status observed in the present study agrees with the results detected by Blanchard et al., (2009).

On comparing claudin 4 expression in TNBC versus non-TNBC cases a significant difference was found. The majority of triple negative cases showed high claudin-4 expression in 75% of cases versus 46.7% in non-TNBC cases. Previous studies reported high claudin-4 expression scores in 66.1% and 62.5% of TNBC cases (Abd-Elazeem and Abd-Elazeem., 2015; Ahmed 2017, respectively). Moreover, Kulka et al., (2009) found that claudin-4 expression was significantly higher in the basal-like compared with the non-basal-like carcinomas and suggested that basal-like carcinomas are a subset of BC with high level of claudin-4 protein expression.

On the other hand, Logullo and his colleagues reported a statistically significant differences in

claudin-4 expression among the different molecular subtypes where high claudin-4 expression scores were less expressed in the luminal and triple-negative subtypes while exhibited the highest frequency in HER-2-enriched tumors (Logullo 2018). The lower expression of claudin-4 among TNBC cases in the previously mentioned study was explained that a subset of the TNBC may undergo epithelial-to-mesenchymal transition phenomenon.

Claudin-4 mediated tumor cell invasion and migration in different cancers (Kwon 2013; Ma 2015; Hicks 2016). To the best of our knowledge, little is known regarding claudin-4 expression in primary BC cases and their corresponding metastatic sites, either regional LNs or distant metastasis. To furthermore investigate this issue, we evaluated claudin-4 expression in 12 pairs of primary IDC-NST cases and their corresponding LN metastasis. It was found that claudin-4 was frequently maintained during metastatic dissemination from primary tumors to their matched LN metastases with a concordance rate of 75%. This finding suggests that claudin-4 bearing BC cells have more ability to metastasize to LNs and supports the notion that claudin-4 expression may confer survival advantage of metastatic cells within LN tissue, potentially by promoting cellular cohesion.

The current study suggests claudin-4 as a molecular protein with an oncogenic function and biological value. Being involved in IDC-NST progression and its association with poor prognostic features including high tumor grade, advanced tumor stage, LN metastasis, high LNR, poor NPI high Ki67 PI and triple negative phenotype highlights its role in BC progression and spread.

References

1. Abd-Elazeem, M. A., & Abd-Elazeem, M. A. (2015). Claudin 4 expression in triple-negative breast cancer: correlation with androgen receptors and Ki-67 expr-ession. Annals of diagnostic pathology, 19(1), 37-42.
2. Adly, S., Hewedi, I. H., & Mokhtar, N. M. (2010). Clinicopathologic significance of molecular classification of breast cancer: relation to nottingham prognosis index. Journal of the Egyptian National Cancer Institute, 22(4), 209-215.
3. Ahmed, S., Harb, O. A., & Nawar, N. (2017). Prognostic Implications of Claudin 4 and Rock 1 in Triple Negative Breast Cancer. Journal of Cancer Treatment and Research, 5(6), 95.
4. Blanchard, A. A., Skliris, G. P., Watson, P. H., Murphy, L. C., Penner, C., Tomes, L., ... & Myal, Y. (2009). Claudins 1, 3, and 4 protein expression in ER negative breast cancer correlates with markers of the basal phenotype. Virchows Archiv, 454(6), 647-656.
5. Cheng, S. A., Liang, L. Z., Liang, Q. L., Huang, Z. Y., Peng, X. X., Hong, X. C., ... & Jiang, L. (2018). Breast cancer laterality and molecular subtype likely share a common risk factor. Cancer management and research, 10, 6549.
6. Cunningham, S. C., Kamangar, F., Kim, M. P., Hammoud, S., Haque, R., Iacobuzio-Donahue, C. A., ... & Choti, M. A. (2006). Claudin-4, mitogen-activated protein kinase kinase 4, and stratifin are markers of gastric adenocarcinoma precursor lesions. Cancer Epidemiology and Prevention Biomarkers, 15(2), 281-287.
7. Ding, L., Lu, Z., Lu, Q., & Chen, Y. H. (2013). The claudin family of proteins in human malignancy: a clinical perspective. Cancer management and research, 5, 367.
8. Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D. M., Piñeros, M., ... & Bray, F. (2019). Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. International journal of cancer, 144(8), 1941-1953.
9. Fernando, A., Jayarajah, U., Prabashani, S., Fernando, E. A., & Seneviratne, S. A. (2018). Incidence trends and patterns of breast cancer in Sri Lanka: an analysis of the national cancer database. BMC cancer, 18(1), 482.
10. Gowrikumar, S., Singh, A. B., & Dhawan, P. (2019). Role of Claudin Proteins in Regulating Cancer Stem Cells and Chemo-resistance-Potential Implication in Disease Prognosis and Therapy. Interna-tional Journal of Molecular Sciences,21(1), 53.
11. Hicks, D. A., Galimanis, C. E., Webb, P. G., Spillman, M. A., Behbakht, K., Neville,

M. C., & Baumgartner, H. K. (2016). Claudin-4 activity in ovarian tumor cell apoptosis resistance and migration. BMC cancer, 16(1), 788.
12. Katayama, A., Handa, T., Komatsu, K., Togo, M., Horiguchi, J., Nishiyama, M., & Oyama, T. (2017). Expression patterns of claudins in patients with triple-negative breast cancer are associated with nodal metastasis and worse outcome. Pathology international, 67(8), 404-413.
13. Kominsky, S. L., Vali, M., Korz, D., Gabig, T. G., Weitzman, S. A., Argani, P., & Sukumar, S. (2004). Clostridium perfringens enterotoxin elicits rapid and specific cytolysis of breast carcinoma cells mediated through tight junction proteins claudin 3 and 4. The American journal of pathology, 164(5), 1627-1633.
14. Konecny, G. E., Agarwal, R., Keeney, G. A., Winterhoff, B., Jones, M. B., Mariani, A., ... & Morin, P. J. (2008). Claudin-3 and claudin-4 expression in serous papillary, clear-cell, and endometrioid endometrial cancer. Gynecologic oncology, 109(2), 263-269.
15. Kulka, J., Szász, A. M., Németh, Z., Madaras, L., Schaff, Z., Molnár, I. A., & Tőkés, A. M. (2009). Expression of tight junction protein claudin-4 in basal-like breast carcinomas. Pathology & Oncology Research, 15(1), 59-64.
16. Kwon, M. J. (2013). Emerging roles of claudins in human cancer. International journal of molecular sciences, 14(9), 18148-18180.
17. Lanigan, F., McKiernan, E., Brennan, D. J., Hegarty, S., Millikan, R. C., McBryan, J., ... & Gallagher, W. M. (2009). Increased claudin-4 expression is associated with poor prognosis and high tumour grade in breast cancer. International Journal of Cancer, 124(9), 2088-2097.
18. Logullo, A. F., Pasini, F. S., Nonogaki, S., Rocha, R. M., Soares, F. A., & Brentani, M. M. (2018). Immunoexpression of claudins 4 and 7 among invasive breast carcinoma subtypes: A large diagnostic study using tissue microarray. Molecular and Clinical Oncology, 9(4), 377-388.
19. Lu, S., Singh, K., Mangray, S., Tavares, R., Noble, L., Resnick, M. B., & Yakirevich, E. (2013). Claudin expression in high-grade invasive ductal carcinoma of the breast: correlation with the molecular subtype. Modern pathology, 26(4), 485
20. Ma, F., Ding, X., Fan, Y., Ying, J., Zheng, S., Lu, N., & Xu, B. (2014). A CLDN1-negative phenotype predicts poor prognosis in triple-negative breast cancer. PLoS One, 9(11), e112765.
21. Ma, X., Miao, H., Jing, B., Pan, Q., Zhang, H., Chen, Y., ... & Li, M. (2015). Claudin-4 controls the proliferation, apoptosis, migration and in vivo growth of MCF-7 breast cancer cells. Oncology reports, 34(2), 681-690.
22. Mineta, K., Yamamoto, Y., Yamazaki, Y., Tanaka, H., Tada, Y., Saito, K., … Tsukita, S. (2011). Predicted expansion of the claudin multigene family. FEBS Letters, 585(4), 606–612.
23. Nichols, L. S., Ashfaq, R., & Iacobuzio-Donahue, C. A. (2004). Claudin 4 protein expression in primary and metastatic pancreatic cancer: support for use as a therapeutic target. American journal of clinical pathology, 121(2), 226-230.
24. Sheehan, G. M., Kallakury, B. V., Sheehan, C. E., Fisher, H. A., Kaufman Jr, R. P., & Ross, J. S. (2007). Loss of claudins-1 and-7 and expression of claudins-3 and-4 correlate with prognostic variables in prostatic adenocarcinomas. Human pathology, 38(4), 564-569.
25. Tőkés, A. M., Kulka, J., Paku, S., Szik, Á., Páska, C., Novák, P. K., ... & Schaff, Z. (2005). Claudin-1,-3 and-4 proteins and mRNA expression in benign and malignant breast lesions: a research study. Breast Cancer Research, 7(2), R296-305.
26. Zardavas, D., Irrthum, A., Swanton, C., & Piccart, M. (2015). Clinical management of breast cancer heterogeneity. Nature Reviews Clinical Oncology, 12(7), 381–394.