JCP Online First, published on February 29, 2016 as 10.1136/jclinpath-2015-203265

Prognostic significance of Claudin 12 in estrogen receptor-negative breast cancer

Omid Iravani,[1] George Wai-Cheong Yip,[1] Aye Aye Thike,[2] Pei Jou Chua,[1] Olivia Jane Scully,[1] Puay-Hoon Tan,[1,2] Boon-Huat Bay[1]

[1]Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[2]Department of Pathology, Singapore General Hospital, Singapore, Singapore

Correspondence to
Dr Omid Iravani, Department of Anatomy, Centre for Translational Medicine (CeTM), Yong Loo Lin School of Medicine, National University of Singapore, MD6, 14 Medical Drive, Singapore 117599, Singapore; antoi@nus.edu.sg

Received 13 July 2015
Revised 30 January 2016
Accepted 3 February 2016

ABSTRACT

Aims Altered expression of the Claudin (CLDN) superfamily of tight junction proteins has been reported in breast cancer. The aim of this study was to examine the immunohistochemical expression of CLDN 12 and its prognostic significance in breast cancer tissues.

Methods Immunohistochemical expression of CLDN 12 was performed on tissue microarrays consisting of 232 cases of breast carcinoma and correlated with clinicopathological features as well as survival of the patients with breast cancer.

Results For the estrogen receptor (ER)-negative subgroup of patients with breast cancer, CLDN 12 expression was shown to be an independent predictor of poor overall survival (HR=2.345; p=0.020) and disease-free survival (HR=2.177; p=0.026) but not for the ER-positive tumours.

Conclusions The findings suggest that CLDN 12 expression could be clinically useful for predicting the survival of the ER-negative subgroup of patients with breast cancer.

INTRODUCTION

Globally, breast cancer is ranked fifth in 2012 for cancer mortality in both genders.[1] As the most frequently diagnosed malignancy among women in the world, it is also the leading cause of female cancer deaths in less-developed nations and ranks second for cancer mortality in developed countries (after lung cancer).[2] Among Chinese women, breast cancer is currently the most common malignancy with a rising incidence during the last decade. Due to the demographic characteristics, population size, geographical condition, socioeconomic factors and reproduction policies, the onset of occurrence of this malignancy has increased in younger Chinese women.[3] Although breast cancer may have a heterogeneous nature among different populations, the age-specific incidence rate of breast cancer among females living in Singapore, China, Hong Kong, Taiwan and South Korea was observed to be more or less similar to the USA.[4]

According to Annual Cancer Registry in Singapore, breast cancer is the most frequently diagnosed malignancy in females (29.3%). It also has the highest rate of cancer mortality among females in Singapore (18.1%). Chinese females in Singapore have been reported to be at a higher risk for developing breast cancer at an earlier age than Malay and Indian residents. However, the 5-year survival of the Chinese female afflicted with breast cancer was longer than affected females in the two other ethnic groups.[5] During the last decade, notable improvements have been made in terms of early diagnosis and treatment of breast cancer, but it still remains as a major cause of cancer mortality among females.[2 6 7] Although clinical and histopathological examinations are usually used for definitive diagnosis, molecular biomarkers have been evaluated for prognostication of patients with cancer and also as potential therapeutic targets. Therefore, identifying novel biomarkers is of importance in guiding clinicians to select the appropriate treatment regimen for patients with breast cancer.[8]

Claudins (CLDNs) are a superfamily of tight junction integral membranous proteins comprising at least 27 subtypes.[9] Based on their functions and structure, they are further subdivided into classical (for instance, CLDNs 1–10, 14, 15, 17 and 19) and non-classical CLDNs (such as 11–13, 16, 18 and 20–24).[10] CLDNs are known to function as paracellular barriers for diffusion of solutes between epithelial cells.[11] Apart from their adhesion characteristics, they selectively regulate transport of water, ions and a variety of macromolecules and consequently, maintain the cellular polarity and integrity. Hence, they are involved in a variety of pathological states such as oedema, jaundice, diarrhoea, tumourigenesis and cancer metastasis. Genetic mutations or alterations in the expression of *CLDN* genes may also lead to developmental disorders, morbidity and even mortality.[12–15] Recently, a growing body of literature has focused on the expression of CLDNs and their tumour-promoting role in a variety of cancers.[16 17]

CLDN 12, which is known to be widely expressed in several tissues,[18] has been reported to be dysregulated in malignancies such as colorectal cancer and skin melanoma.[19 20] CLDN 12 is expressed strongly in MeWo and G-361 human melanoma cell lines at the mRNA level. In addition, it has been detected at the intercellular border of melanoma tissues.[20] In colorectal carcinoma, *CLDN 12* was also found to be upregulated at mRNA level.[19] In Crohn's disease, CLDN 12 was shown to be highly expressed in the ileum, but weakly expressed in sigmoid colon.[21] Using in silico analysis, Hewitt *et al*[18] observed a wide dysregulated expression of *CLDN 12* in breast cancer tissues. Recently, Yang *et al*[22] reported that enhanced cell migration in breast cancer cells is associated with downregulation of *CLDN 12*. However, there is still insufficient information regarding the functional role and clinicopathological significance of CLDN 12 in human breast cancer.

The aim of this study was to correlate the immunohistochemical expression of CLDN 12 in breast cancer with clinicopathological features as well as

To cite: Iravani O, Yip GW-C, Thike AA, *et al*. *J Clin Pathol* Published Online First: [*please include* Day Month Year] doi:10.1136/jclinpath-2015-203265

patient survival, which was stratified according to the estrogen receptor (ER) status.

MATERIALS AND METHODS

Patients and samples studied

Paraffin-embedded tissue microarray (TMA) slides (core size: 1 mm) were constructed from archival blocks of a total of 232 cores of breast carcinoma tissues, diagnosed between 1998 and 2006 and collected by the Department of Pathology of Singapore General Hospital. All specimens were obtained post-surgery and prior to any other therapy. Using similar specimens, our colleagues previously reported a substantial agreement between TMA specimens and standard sections.[23] Clinicopathological features were collected from medical record of the patients and included race, age, tumour size, histological subtype, histological grade, lymph node status, ER, progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) (table 1). Histological subtype was subdivided into invasive ductal carcinoma (IDC) and non-IDC subtype. Non-IDC subtypes include mixed carcinoma, invasive lobular carcinoma, invasive micropapillary carcinoma, invasive tubular carcinoma, mucinous carcinoma, cribriform carcinoma, medullary carcinoma and metaplastic carcinoma. Information such as the date of diagnosis, recurrence and death were retrieved from patient records. The specimens were taken from patients between 1998 and 2006. The minimum, maximum and mean lengths of follow-up were 0.067, 173.4 and 108.74 months, respectively. Approval of this study was granted by the Institutional Review Board of the Singapore General Hospital.

Table 1 Clinicopathological features of patients with breast cancer (n=232)

Characteristics	No	Per cent
Age		
≤57 (mean)	130	56
>57 (mean)	102	44
Race		
Chinese	163	70.3
Malay	14	6.0
Indians	8	3.4
Others	9	3.9
Unavailable	38	16.4
Tumour size		
pT1-2	193	83.2
pT3-4	35	15.1
Unavailable	4	1.7
Histological grade		
1–2	110	47.4
3	120	51.7
Unavailable	2	0.9
Histological subtype		
IDC	205	88.4
Non-IDC	27	11.6
Lymph node status		
Negative	105	45.3
Positive	74	31.9
Unavailable	53	22.8
ER		
Negative	122	52.6
Positive	110	47.4
Unavailable		
PR		
Negative	95	40.9
Positive	136	58.6
Unavailable	1	0.4
HER-2		
Negative	154	66.4
Positive	75	32.3
Unavailable	3	1.3

ER, estrogen receptor; HER-2, human epidermal growth receptor 2; IDC, invasive ductal carcinoma; PR, progesterone receptor.

Immunohistochemical staining

Four-micrometer thick paraffin-embedded TMA sections were deparaffinised and then rehydrated using clearene and a graded series of ethanol. After treating with 3% hydrogen peroxide (to suppress endogenous peroxidase activity) for 30 min, the tissue sections were then blocked with 1% normal goat serum in Tris-buffered saline at pH 7.6 for 60 min. After which, slides were incubated overnight at 4°C with rabbit polyclonal CLDN 12 antibody (dilution, 1:50; Sigma, USA). The antibodies for ER (SP1, RM9101-S, NeoMarkers), PR (PgR636, RM9102-S, NeoMarkers) and HER-2 (SP3, RM9103-S, NeoMarkers) were used at dilutions of 1:50, 1:200 and 1:200 respectively. Secondary antibody was added according to the protocol in the Avidin–Biotin Complex kit (Vector Laboratories, USA). Sections were then exposed to diaminobenzidine as a chromogen substrate for 20 min. Haematoxylin counterstaining was performed and tissue sections were finally mounted, examined and scored by a pathologist. Definitive staining of endothelial cells in the breast cancer specimens were used as internal positive controls. The percentage of staining of the epithelial cells was noted and staining intensity determined as strong staining (3+), moderate staining (2+), weak staining (1+) to no staining (0). H-score was calculated by the formula: (1× % weak staining)+(2× % moderate staining)+(3×% strong staining). The staining intensities were verified by a pathologist. As CLDN 12 expression was mainly low to moderate, the groups were stratified according to a cut-off level of 20, which is approximately located within the lowest 25th percentile of the H-score (consisting of 71.6% of the breast cancer cases). A cut-off based on values approximating within the lowest 25 percentile of the staining intensity has been used in several studies.[24 25] For ER and PR positivity, a cut-off of at least 1% nuclear staining was used, whereas for HER-2 positivity, the cut-off was minimum 30% 3 + membrane staining.[26 27]

Statistical analysis

Statistical analysis was done with the GraphPad Prism 5.0 and PASW (SPSS) Statistics 18. Fisher's exact test was used to find associations between the CLDN 12 staining and clinicopathological features. The Kaplan–Meier method was used to perform survival analysis. Multivariate Cox regression analysis was performed to measure HR within 95% CI using backward stepwise model. $p<0.05$ was considered statistically significant.

RESULTS

Clinicopathological features of the studied population

The age of the patients with breast cancer ranged from 23 to 89 years, with a mean age of 56.8 years. Tumour size ranged from 5 to 140 mm with a mean size of 34.71 mm. Recurrence occurred in 65 (28%) cases, while 49 (21.1%) patients died during the follow-up period. The mean overall survival (OS; length of time from the date of diagnosis to death) and disease-free survival (DFS; period of time from diagnosis to recurrence)

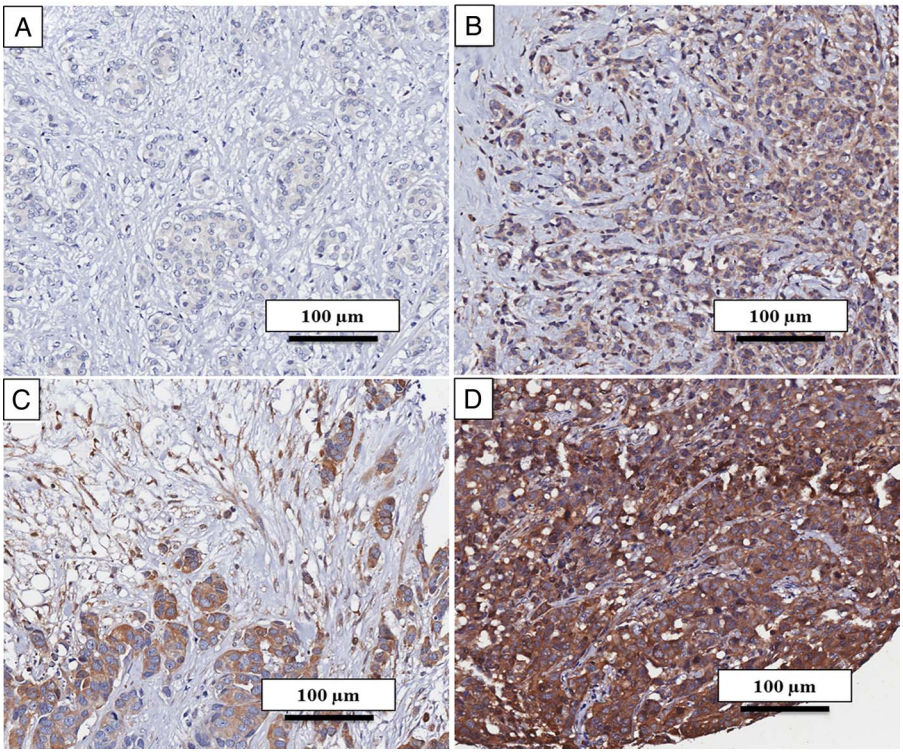

Figure 1 Immunohistochemical analysis of Claudin (CLDN) 12 in breast cancer sections depicting: (A) no staining (0); (B) weak staining (1+); (C) moderate staining intensity (2+) and (D) strong staining intensity (3+); bar=100 µm.

of the patients were 108.7 and 103.02 months, respectively. The clinicopathological features are summarised in table 1.

Correlation of clinicopathological features with CLDN 12

The CLDN 12 protein was observed to be mainly localised in the cytoplasm of breast cancer epithelial cells (figure 1). CLDN 12 exhibited a low-to-moderate cytoplasmic expression in the breast cancer sections with mean H-score of 18.90. In adjacent benign tissues, cells of normal breast ducts were observed to have weak cytoplasmic staining (not shown). There was no significant correlation observed between CLDN 12 immunostaining and clinicopathological features when stratified into ER-negative and ER-positive subgroups of breast cancer (table 2). In addition, expression of the CLDN 12 protein in the ER-negative subgroup did not significantly differ from the ER-positive subgroup (p=0.685).

CLDN 12 expression and survival of the patients with breast cancer

Kaplan–Meier survival analysis revealed that the expression of CLDN 12 was significantly associated with OS and DFS in patients with ER-negative breast cancer (figure 2). For the ER-negative subgroup of patients with breast cancer, univariate analysis showed that age, tumour size and CLDN 12 expression were associated with OS, while age, tumour size, histological grade and CLDN 12 expression with DFS (table 3). Older patients, larger tumour size, higher histological grades and increased CLDN12 immunostaining were found to be associated with poorer survival in patients with ER-negative breast cancer.

As shown in table 4, multivariate analysis revealed that the expression of CLDN 12 is an independent predictor of poor OS (HR=2.345; p=0.020) and DFS (HR=2.177; p=0.026) in patients with ER-negative breast cancer. The multivariate analysis was performed by including the age, tumour size and H-score for OS and age, tumour size, histological grade and H-score for DFS.

Interestingly, for the ER-positive subgroup of patients with breast cancer, CLDN 12 immunostaining was not predictive for OS or DFS although positive lymph node status was associated with a poorer DFS (table 5).

DISCUSSION

Recently, several studies have focused on the role of CLDN family members in the tumourigenesis as well as prognosis of

Table 2 Correlation between clinicopathological parameters and CLDN 12 immunoreactivity

	H-score (ER-negative)			H-score (ER-positive)		
	≤20	>20	p Value	≤20	>20	p Value
Age						
≤57 (mean)	51	15	0.410	43	21	0.679
>57 (mean)	39	17		33	13	
Tumour size						
pT1-2	76	24	0.155	65	28	1
pT3-4	11	8		11	5	
Histological grade						
Grade 1–2	38	10	0.397	43	19	1
Grade 3	52	21		33	14	
Lymph node status						
Negative	38	13	0.639	34	20	0.653
Positive	27	12		24	11	
PR						
Negative	55	24	0.201	13	3	0.382
Positive	34	8		63	31	
HER-2						
Negative	54	17	0.830	59	24	0.476
Positive	35	13		17	10	

CLDN, Claudin; ER, estrogen receptor; PR, progesterone receptor.

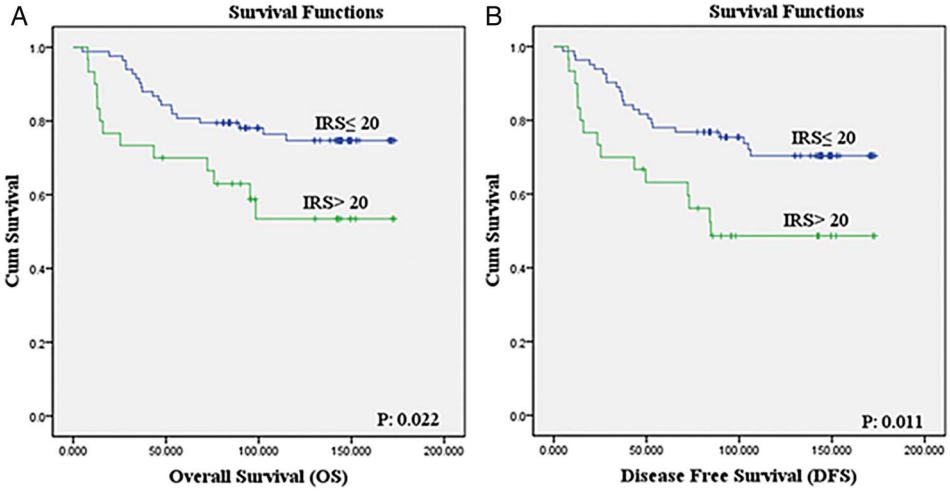

Figure 2 Kaplan–Meier survival analysis revealed that the expression of Claudin (CLDN) 12 was predictive for overall survival (OS) and disease-free survival (DFS) in estrogen receptor (ER)-negative breast cancer, *p<0.05 (p value was retrieved from log-rank test).

Table 3 Univariate Cox regression analysis for clinicopathological factors associated with OS and DFS in patients with ER-negative breast cancer

	Overall survival			Disease-free survival		
Predictor	**HR**	**95% CI**	**p Value**	**HR**	**95% CI**	**p Value**
Age						
≤57 (mean) vs >57 (mean)	2.244	1.113 to 4.427	0.024*	1.961	1.027 to 3.745	0.041*
Tumour size						
pT1-2 vs pT3-4	2.664	1.223 to 5.805	0.014*	2.143	1.005 to 4.568	0.049*
Histological grade						
Grade 1–2 vs 3	1.837	0.848 to 3.977	0.123	2.068	0.999 to 4.281	0.050*
Lymph node status						
Negative vs positive	2.396	0.992 to 5.786	0.052	1.865	0.846 to 4.113	0.122
PR						
Negative vs positive	0.996	0.489 to 2.028	0.991	1.126	0.587 to 2.159	0.722
HER-2						
Negative vs positive	0.541	0.257 to 1.136	0.105	0.554	0.280 to 1.099	0.091
CLDN 12 (H-score)						
≤20 vs >20	2.222	1.104 to 4.472	0.025*	2.275	1.184 to 4.369	0.014*

*p<0.05.
CLDN, Claudin; DFS, disease-free survival; ER, estrogen receptor; OS, overall survival; PR, progesterone receptor.

breast cancer.[28 29] CLDN 1, 2 and 7 expressions have been reported to be downregulated,[30–32] while CLDN 3 and 4 found to be upregulated in breast cancer.[33] The implications of CLDN expression in breast cancer is evident, as a molecular subtype of breast cancer expressing low level of CLDN 3, 4 and 7 has been classified as CLDN-low breast cancer.[34] This subgroup of breast cancer has been observed to have a poor prognosis and comprise 12–14% of breast malignancies. Lu *et al*[35] also observed that a low level of expression of CLDN 1, 3, 4, 7 and 8 was associated with more recurrences in breast cancer. Although the clinical importance of many CLDNs has not been characterised, expression of CLDN 1 and 4 has been reported to have prognostic values in breast cancer.[36 37]

In the current study, it was shown that CLDN 12 is expressed in the cytoplasm of breast cancer cells. CLDN family members theoretically have a subcellular membrane-specific localisation. Each of the CLDN family members structurally consists of four transmembrane N-terminal domains and a small C-terminal domain in the cytoplasm. However, membranous expression of some of these tight junction proteins may be disturbed in some

Table 4 Multivariate Cox regression analysis for prognostic factors associated with OS and DFS in patients with ER-negative breast cancer

Predictor	**HR**	**95% CI**	**p Value**
Overall survival			
Tumour size			
pT1–2 vs pT3–4	2.579	1.180 to 5.635	0.018*
CLDN 12 (H-score)			
≤20 vs >20	2.345	1.145 to 4.803	0.020*
Disease-free survival			
Tumour size			
pT1-2 vs pT3-4	2.234	1.038 to 4.810	0.040*
Histological grade			
Grade 1–2 vs 3	2.253	1.051 to 4.832	0.037*
CLDN 12 (H-score)			
≤20 vs >20	2.177	1.097 to 4.319	0.026*

*p<0.05.
CLDN, Claudin; DFS, disease-free survival; ER, estrogen receptor; OS, overall survival.

Table 5 Univariate Cox regression analysis for the clinicopathological factors associated with OS and DFS in patients with ER-positive breast cancer

	Overall survival			Disease-free survival		
Predictor	**HR**	**95% CI**	**p Value**	**HR**	**95% CI**	**p Value**
Age						
≤57 (mean) vs >57 (mean)	1.515	0.568 to 4.041	0.406	1.087	0.499 to 2.368	0.833
Tumour size						
pT1-2 vs pT3-4	1.782	0.506 to 6.271	0.368	1.835	0.691 to 4.876	0.223
Histological grade						
Grade 1–2 vs 3	0.953	0.353 to 2.573	0.924	1.103	0.508 to 2.395	0.804
Lymph node status						
Negative vs positive	1.758	0.616 to 5.023	0.292	2.385	1.043 to 5.453	0.039*
PR						
Negative vs positive	25.563	0.057 to 11 439.620	0.298	25.738	0.228 to 2909.125	0.178
HER-2						
Negative vs positive	1.038	0.334 to 3.224	0.949	1.432	0.622 to 3.300	0.399
CLDN 12 (H-score)						
≤20 vs >20	0.632	0.204 to 1.962	0.427	1.025	0.456 to 2.301	0.953

*p<0.05.
CLDN, Claudin; DFS, disease-free survival; ER, estrogen receptor; OS, overall survival; PR, progesterone receptor.

malignant conditions. For example, cytoplasmic mislocalisation of CLDN 1 was reported in breast cancer.[38] This expression pattern has not been well studied; however, it might be due to disturbance in transportation of tight junction proteins to the cell membrane. In this scenario, cytoplasmic accumulation of these proteins is expected.[39]

Although there was no correlation observed between CLDN 12 immunostaining and clinicopathological parameters examined, clustering of the samples into two subgroups of ER-negative and ER-positive breast cancers revealed that CLDN 12 immunostaining was an independent predictor of a worse OS and DFS in patients with ER-negative breast cancer, but not in patients with ER-positive breast cancer. It would therefore appear that CLDN 12 may behave differently in the two subgroups of breast cancer. ER-negative breast cancers are a subgroup of breast cancer where younger females are known to be at a higher risk for developing an aggressive type.[40] The patients have a poorer short-term survival in this same study; however, the long-term (>5 years) recurrence rate is low.[40]

Although the incidence of the ER−/PR+ breast cancer phenotype is not frequent, approximately 18% cases were identified in this present study. Reports have shown that the occurrence of the ER−/PR+ breast cancer phenotype is different among the various ethnic groups, such as that observed by Chu *et al*.[41] Similarly, Kuzhan and colleagues observed a significantly different distribution of the hormone receptor status among patients with breast cancer from different ethnic groups in Turkey.[42] A high percentage of the ER−/PR+ phenotype was also found among patients with breast cancer from Chinese (10%) and Indian (21%) populations.[43 44] Thus, it would appear that ethnicity is a critical factor for hormone receptor status in breast cancer.

CLDN 12 was reported to be frequently upregulated at mRNA level in colorectal carcinoma. More than 40% of the colorectal cancer tissues were observed to be upregulated by more than twofolds for *CLDN 12*.[19] A high expression of CLDN 12 was clearly present on the cell membranes of melanoma cells.[20] Yang *et al*[22] showed that siRNA-mediated silencing of *CLDN 12* increased cell migration in MDA-MB231 and MCF7 cell lines. The results of the present TMA study are not consistent with that reported by Yang *et al*, possibly because of the different study methods used. The advantage of tissue studies as opposed to in vitro studies is that the former takes into account the tissue microenvironment, which has a profound influence during breast carcinogenesis.[45]

To our knowledge, this is the first time that CLDN 12 has been found to be a significant predictive biomarker for poor OS and DFS in ER-negative breast cancer. One of the most challenging aspects of breast cancer therapy has been the lack of ER targets for therapeutic intervention.[40] Thus, identifying novel targets in ER-negative breast cancer could lead to novel therapeutic strategies. Developing novel molecular markers for prognostication purposes would be valuable especially when traditional prognostics are unable to predict the clinical outcome of the disease.[46] More investigations would be required to explore CLDN 12 as a potential therapeutic target in this subtype of breast cancers.

Take home messages

- *CLDN 12* is differentially expressed in breast cancer tissues.
- CLDN 12 is mainly expressed in the cytoplasm of the breast cancer cells, with low-to-moderate expression.
- CLDN 12 expression in the cancer tissues of patients with ER-negative breast cancer is predictive of a worse overall and disease-free survival.

Handling editor Cheok Soon Lee

Contributors OI: conception, immunohistochemical staining, data analysis, drafting and finalisation of manuscript; GW-CY, PJC, OJS, P-HT: interpretation of data and amendments to manuscript; AAT: verification of immunohistochemical staining data; B-HB: conception, interpretation of data, amendments and finalisation of manuscript.

Funding This work was supported by Grants BMRC/10/1/21/24/638 and NMRC/CIRG/1370/2013.

Competing interests None declared.

Patient consent Obtained.

Ethics approval This study was approved by the Institutional Review Board of the Singapore General Hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Ferlay J, Soerjomataram I, Dikshit R, *et al*. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359–86.
2 Torre LA, Bray F, Siegel RL, *et al*. Global cancer statistics, 2012. *CA Cancer J Clin* 2015;65:87–108.
3 Fan L, Strasser-Weippl K, Li JJ, *et al*. Breast cancer in China. *Lancet Oncol* 2014;15: e279–89.
4 Sung HN, Rosenberg PS, Chen WQ, *et al*. Female breast cancer incidence among Asian and Western Populations: more similar than expected. *Jnci-J Natl Cancer I* 2015;107:djv107.
5 Singapore Cancer Registry. Trends in Cancer Incidence in Singapore 2009–2013. Annual Registry Report, 2015. https://www.nrdo.gov.sg/docs/librariesprovider3/Publications-Cancer/cancer-trends-report-2009-2013.pdf?sfvrsn=0 (accessed 8 Dec 2015).
6 Cancer facts and figures. 2015. http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf (accessed 8 Dec 2015).
7 Jemal A, Center MM, DeSantis C, *et al*. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 2010;19:1893–907.
8 Cole KD, He HJ, Wang L. Breast cancer biomarker measurements and standards. *Proteomics Clin Appl* 2013;7:17–29.
9 Mineta K, Yamamoto Y, Yamazaki Y, *et al*. Predicted expansion of the claudin multigene family. *FEBS Lett* 2011;585:606–12.
10 Krause G, Winkler L, Piehl C, *et al*. Structure and function of extracellular claudin domains. *Ann N Y Acad Sci* 2009;1165:34–43.
11 Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol* 2001;2:285–93.
12 Krug SM, Günzel D, Conrad MP, *et al*. Charge-selective claudin channels. *Ann N Y Acad Sci* 2012;1257:20–8.
13 Furuse M, Moriwaki K. The role of claudin-based tight junctions in morphogenesis. *Ann N Y Acad Sci* 2009;1165:58–61.
14 Sawada N, Murata M, Kikuchi K, *et al*. Tight junctions and human diseases. *Med Electron Microsc* 2003;36:147–56.
15 Swisshelm K, Macek R, Kubbies M. Role of claudins in tumorigenesis. *Adv Drug Deliv Rev* 2005;57:919–28.
16 Lourenço SV, Coutinho-Camillo CM, Buim ME, *et al*. Claudin-7 down-regulation is an important feature in oral squamous cell carcinoma. *Histopathology* 2010;57:689–98.
17 Escudero-Esparza A, Jiang WG, Martin TA. The Claudin family and its role in cancer and metastasis. *Front Biosci (Landmark Ed)* 2011;16:1069–83.
18 Hewitt KJ, Agarwal R, Morin PJ. The claudin gene family: expression in normal and neoplastic tissues. *BMC Cancer* 2006;6:186.
19 Gröne J, Weber B, Staub E, *et al*. Differential expression of genes encoding tight junction proteins in colorectal cancer: frequent dysregulation of claudin-1, -8 and -12. *Int J Colorectal Dis* 2007;22:651–9.
20 Morita K, Morita NI, Nemoto K, *et al*. Expression of claudin in melanoma cells. *J Dermatol* 2008;35:36–8.
21 Lameris AL, Huybers S, Kaukinen K, *et al*. Expression profiling of claudins in the human gastrointestinal tract in health and during inflammatory bowel disease. *Scand J Gastroenterol* 2013;48:58–69.
22 Yang Y, Cheon S, Jung MK, *et al*. Interleukin-18 enhances breast cancer cell migration via down-regulation of claudin-12 and induction of the p38 MAPK pathway. *Biochem Biophys Res Commun* 2015;459:379–86.
23 Selvarajan S, Tan SY, Sii LH, *et al*. c-erbB-2 (HER-2/neu) immunohistochemistry in invasive breast cancer: is there concordance between standard sections and tissue microarrays? *Pathology* 2006;38:316–20.
24 Skliris GP, Nugent ZJ, Rowan BG, *et al*. A phosphorylation code for oestrogen receptor-alpha predicts clinical outcome to endocrine therapy in breast cancer. *Endocr Relat Cancer* 2010;17:589–97.
25 Skliris GP, Hubé F, Gheorghiu I, *et al*. Expression of small breast epithelial mucin (SBEM) protein in tissue microarrays (TMAs) of primary invasive breast cancers. *Histopathology* 2008;52:355–69.
26 Idirisinghe PK, Thike AA, Cheok PY, *et al*. Hormone receptor and c-ERBB2 status in distant metastatic and locally recurrent breast cancer. Pathologic correlations and clinical significance. *Am J Clin Pathol* 2010;133:416–29.
27 Shah S, Chen B. Testing for HER2 in breast cancer: a continuing evolution. *Patholog Res Int* 2011;2011:903202.
28 Ricardo S, Gerhard R, Cameselle-Teijeiro JF, *et al*. Claudin expression in breast cancer: high or low, what to expect? *Histol Histopathol* 2012;27:1283–95.
29 Tokés AM, Kulka J, Paku S, *et al*. Claudin-1, -3 and -4 proteins and mRNA expression in benign and malignant breast lesions: a research study. *Breast Cancer Res* 2005;7:R296–305.
30 Kominsky SL, Argani P, Korz D, *et al*. Loss of the tight junction protein claudin-7 correlates with histological grade in both ductal carcinoma in situ and invasive ductal carcinoma of the breast. *Oncogene* 2003;22:2021–33.
31 Kim TH, Huh JH, Lee S, *et al*. Down-regulation of claudin-2 in breast carcinomas is associated with advanced disease. *Histopathology* 2008;53:48–55.
32 Krämer F, White K, Kubbies M, *et al*. Genomic organization of claudin-1 and its assessment in hereditary and sporadic breast cancer. *Hum Genet* 2000;107:249–56.
33 Kominsky SL, Vali M, Korz D, *et al*. Clostridium perfringens enterotoxin elicits rapid and specific cytolysis of breast carcinoma cells mediated through tight junction proteins claudin 3 and 4. *Am J Pathol* 2004;164:1627–33.
34 Eroles P, Bosch A, Pérez-Fidalgo JA, *et al*. Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. *Cancer Treat Rev* 2012;38:698–707.
35 Lu S, Singh K, Mangray S, *et al*. Claudin expression in high-grade invasive ductal carcinoma of the breast: correlation with the molecular subtype. *Mod Pathol* 2013;26:485–95.
36 Szasz AM, Tokes AM, Micsinai M, *et al*. Prognostic significance of claudin expression changes in breast cancer with regional lymph node metastasis. *Clin Exp Metastasis* 2011;28:55–63.
37 Lanigan F, McKiernan E, Brennan DJ, *et al*. Increased claudin-4 expression is associated with poor prognosis and high tumour grade in breast cancer. *Int J Cancer* 2009;124:2088–97.
38 Blanchard AA, Ma X, Dueck KJ, *et al*. Claudin 1 expression in basal-like breast cancer is related to patient age. *BMC Cancer* 2013;13:268.
39 Myal Y, Leygue E, Blanchard AA. Claudin 1 in breast tumorigenesis: revelation of a possible novel "claudin high" subset of breast cancers. *J Biomed Biotechnol* 2010;2010:956897. doi: 10.1155/2010/956897
40 Barcellos-Hoff MH. Does microenvironment contribute to the etiology of estrogen receptor-negative breast cancer? *Clin Cancer Res* 2013;19:541–8.
41 Chu KC, Anderson WF, Fritz A, *et al*. Frequency distributions of breast cancer characteristics classified by estrogen receptor and progesterone receptor status for eight racial/ethnic groups. *Cancer* 2001;92:37–45.
42 Kuzhan A, Adli M, Eryigit Alkis H, *et al*. Hormone receptor and HER2 status in patients with breast cancer by races in southeastern Turkey. *J BUON* 2013;18:619–22.
43 Adams SA, Matthews CE, Hebert JR, *et al*. Association of physical activity with hormone receptor status: the Shanghai Breast Cancer Study. *Cancer Epidemiol Biomarkers Prev* 2006;15:1170–8.
44 Desai SB, Moonim MT, Gill AK, *et al*. Hormone receptor status of breast cancer in India: a study of 798 tumours. *Breast* 2000;9:267–70; discussion 270.
45 Ma XJ, Dahiya S, Richardson E, *et al*. Gene expression profiling of the tumor microenvironment during breast cancer progression. *Breast Cancer Res* 2009;11:R7.
46 Liu JG, Li SY, Dunker AK, *et al*. Molecular profiling: an essential technology enabling personalized medicine in breast cancer. *Curr Drug Targets* 2012;13:541–54.

Prognostic significance of Claudin 12 in estrogen receptor-negative breast cancer

Omid Iravani, George Wai-Cheong Yip, Aye Aye Thike, Pei Jou Chua, Olivia Jane Scully, Puay-Hoon Tan and Boon-Huat Bay

J Clin Pathol published online February 29, 2016