Plasma soluble cluster of differentiation 147 levels are increased in breast cancer patients and associated with lymph node metastasis and chemoresistance

YH Kuang, YJ Liu, LL Tang, SM Wang, GJ Yan, LQ Liao *

ABSTRACT

Introduction: Cluster of differentiation 147 (CD147) contributes to breast cancer invasion, metastasis, and multidrug resistance. Recent studies have shown that peripheral soluble CD147 (sCD147) is increased in hepatocellular tumour and multiple myeloma patients and correlated with disease severity. This study aimed to assess the level, as well as the biological and clinical significance of sCD147 in breast cancer.

Methods: We tested plasma sCD147 levels in 308 breast cancer patients by enzyme-linked immunosorbent assay between February 2014 and February 2017. A subset of 165 cases of benign breast diseases was included as control group at the same period. We analysed the clinical significance of plasma sCD147 with relevance to clinicopathological factors of breast cancer patients.

Results: Plasma sCD147 levels were significantly higher in patients with primary breast cancer than those with benign breast diseases (P=0.001), in patients with locally advanced breast cancer (T3-T4 tumour) than those in early breast cancer (T1-T2 tumour; P=0.001), in patients with lymph node metastasis than in those without (P<0.001), and in patients with high recurrence risk than those with medium recurrence risk (P<0.001). Plasma sCD147 levels were also significantly higher in the chemotherapy-resistant group than in the chemotherapy-sensitive group (P=0.040). Plasma sCD147 was an independent predictor for lymph node metastasis in breast cancer patients (P=0.001).

Conclusion: This is the first study to demonstrate that plasma sCD147 levels are elevated in breast cancer patients. Soluble CD147 is also associated with tumour size, lymph node metastasis, high recurrent risk, and chemoresistance. Our findings support that plasma sCD147 is an independent predictive factor for lymph node metastasis.

Hong Kong Med J 2018;24:252–60
DOI: 10.12809/hkmj176865

[1] YH Kuang, PhD
[2] YJ Liu, MSc
[2] LL Tang, PhD
[2] SM Wang, PhD
[2] GJ Yan, BSc
[2] LQ Liao *, PhD

[1] *Department of Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China*
[2] *Department of Breast Surgery, Hunan Clinical Meditech Research Center for Breast Cancer, Xiangya Hospital, Central South University, Changsha, Hunan, China*

* Corresponding author: aq301981@163.com

This article was published on 25 May 2018 at www.hkmj.org.

New knowledge added by this study

- Plasma sCD147 levels are elevated in breast cancer patients and are associated with tumour size, lymph node metastasis, high recurrent risk, and chemoresistance.
- Plasma sCD147 is an independent predictive factor for lymph node metastasis.

Implications for clinical practice or policy

- Plasma sCD147 may be used as the predictive factor to evaluate lymph node metastasis, recurrence risk, and chemoresistance of breast cancer.
- Plasma sCD147 may contribute to the development of optimal adjuvant therapy for individual breast cancer patients.

Introduction

Breast cancer is the most common malignant tumour and the leading cause of cancer-related deaths among females in developing countries.[1] Breast cancer displays heterogeneity: it comprises distinct pathologies and histological features and can have different chemotherapy responses and clinical outcomes.[2] The identification of tumour-related factors that can predict tumour behaviour is important. Predictive factors can help identify as early as possible not only patients who have a high risk of recurrence and metastasis, but also patients

乳腺癌血漿sCD47水平增高促進淋巴結轉移和化療耐藥

匡葉紅、劉瑜婧、唐利立、王守滿、延國姣、廖立秋

引言：CD147促進乳腺癌的侵入、轉移和多藥耐藥。近年研究顯示肝細胞癌和多發性骨髓瘤患者的外周血可溶性CD147（sCD147）水平增加，且與疾病重度相關。本研究旨在探討sCD147在乳腺癌中的表達水平及其生物學和臨床意義。

方法：採用酶聯免疫吸附法檢測2014年2月至2017年2月期間308例乳腺癌患者的血漿sCD147水平，並以同一時期165例良性乳腺疾病的血漿sCD147水平作為對照組。本研究主要分析乳腺癌血漿sCD147水平與乳腺癌患者臨床病理因素的相關性。

結果：原發性乳腺癌患者的血漿sCD147水平顯著高於良性乳腺疾病患者（P=0.001）。局部晚期乳腺癌組（T3-T4腫瘤）的血漿sCD147水平顯著高於早期乳腺癌組（T1-T2腫瘤，P=0.001）。淋巴結轉移患者的血漿sCD147水平顯著高於無淋巴結轉移組（P<0.001）。高復發風險組的血漿sCD147水平顯著高於低復發風險組（P<0.001）。化療耐藥組的血漿sCD147水平顯著高於化療敏感組（P=0.040）。血漿sCD147是乳腺癌淋巴結轉移的獨立預測因子（P=0.001）。

結論：本研究首次發現乳腺癌患者的血漿sCD147水平升高。sCD147水平與腫瘤大小、淋巴結轉移、高復發風險和化療耐藥有關。血漿sCD147是淋巴結轉移的獨立預測因子。

who can benefit from different types of adjuvant therapy.[3] Conventional predictive factors of high risk of recurrence and metastasis include relatively large (>5 cm) tumour size and high nuclear grade; negativity for oestrogen receptor and progesterone receptor; human epidermal growth factor receptor 2 (*HER2*) overexpression; and increased lymph node involvement at the time of breast cancer diagnosis.[4] Recent advances in genetic profiling of tumours have extended our understanding of breast cancer biology and have allowed the use of several prognostic gene signatures to select patients at highest risk of early recurrence and those who may benefit from certain adjuvant treatment.[2,5,6] However, despite receiving standard treatments routinely guided by predictive factors, more than 30% of breast cancer patients develop metastatic disease and have poor survival.[7,8] As such, it is essential and urgent to identify reliable predictive factors to assist in diagnosis, staging, evaluation of recurrence risk, and development of new treatment modalities.

Cluster of differentiation 147 (CD147), a transmembrane glycoprotein that belongs to the immunoglobulin superfamily, can promote tumour invasion and metastasis, and mediate breast cancer drug resistance.[9-13] Expression of CD147 is significantly correlated with axillary lymph node involvement; tumour, node, and metastasis staging; and shorter progression-free survival and overall survival.[14] Previous data demonstrated that CD147 exists in both membrane-bound and soluble forms in many solid tumours, and soluble CD147 (sCD147) can be detected in the conditioned medium of tumour cells and peripheral blood of cancer patients.[15-17] Overexpression of the CD147 gene in human breast cancer cells can increase the sCD147 level, indicating that sCD147 release is correlated with the degree of CD147 expression in tumour cells.[15-17] Full-length CD147 may be exported into the microenvironment from tumour cells by microvesicle shedding or by matrix metalloproteinase (MMP)–dependent cleavage, thereby stimulating MMP expression in fibroblasts.[18-20] In turn, sCD147 derived from tumour cells acts in a paracrine fashion on stromal cells that are both adjacent and distant to tumour sites, so as to further stimulate the production of MMPs and CD147. This additional CD147 consequently contributes to tumour angiogenesis, tumour growth, and metastasis.[16,21] Importantly, several studies investigating the role of sCD147 level in patients with tumours have suggested that sCD147 may offer a useful approach in diagnosis, as it is correlated with disease severity.[15,22] However, little is known about the level of sCD147 in patients with breast cancer. Furthermore, the biological and clinical significance of sCD147 in breast cancer has not been investigated.

In this study, we measured plasma sCD147 levels in patients with breast cancer and evaluated the results with respect to clinicopathological factors. We aimed to demonstrate the association between plasma sCD147 levels with tumour size, lymph node metastasis, recurrence risk, and chemoresistance in breast cancer patients.

Methods

Patients and samples

The results of this study are presented in accordance with the reporting recommendations for tumour marker prognostic studies.[23] We conducted the study between February 2014 and February 2017 in the Affiliated Xiangya Hospital of Central South University in Changsha of Hunan Province, China. We collected peripheral blood samples from consecutive patients with breast cancer, including primary breast cancer, during their first hospital admission. To be eligible for this study, patients had to be adult females who had no other malignant diseases or severe systemic diseases, especially rheumatic, inflammatory, and cardiovascular diseases. The peripheral blood of consecutive patients with palpable benign breast masses, including fibroadenoma and adenopathy, was also collected to serve as control samples during the same period. All blood samples were centrifuged at 3000 rpm at 4°C

for 5 minutes, and the plasma samples were stored at -70°C for later plasma sCD147 testing. All the patients' clinicopathological findings were supplied by the Xiangya Hospital of Central South University. Breast cancer subtypes were identified according to the St Gallen Consensus 2013 classification system.[24] Recurrence risk of breast cancer was evaluated according to the St Gallen Consensus 2007 criteria.[25]

The association between chemotherapy response and plasma sCD147 level was retrospectively analysed. The patients included in this analysis had to meet all of the following criteria: (1) had a confirmed diagnosis of invasive ductal breast carcinoma by pathology and had consented to undergo neoadjuvant chemotherapy; (2) had operable breast cancer consisting of a large tumour (>2 cm) that fulfilled the criteria for breast conserving surgery except tumour size, or triple-negative breast cancer (TNBC; ie, negative for oestrogen/progesterone receptors and *HER2*) with small (T1 stage) tumours; (3) had received no previous treatment; (4) had received only four cycles of pirarubicin-cyclophosphamide/epirubicin-cyclophosphamide (AC/EC)–based neoadjuvant chemotherapy before surgery; and (5) had complete hospital records that included evaluation of chemotherapy efficacy. Clinical response to AC/EC-based chemotherapy was evaluated by the decrease in tumour size and classified according to response evaluation criteria in solid tumours (RECIST criteria).[26] Patients with complete remission or partial remission were classified as chemotherapy-sensitive, whereas patients with stable disease or progressive disease were classified as chemotherapy-resistant.

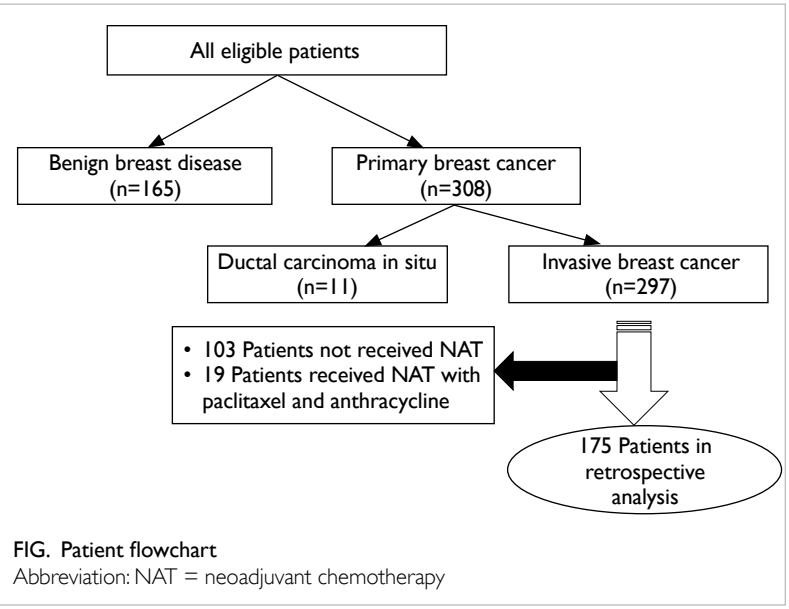

FIG. Patient flowchart
Abbreviation: NAT = neoadjuvant chemotherapy

Enzyme-linked immunosorbent assay

The concentrations of plasma sCD147 were measured by enzyme-linked immunosorbent assay (ELISA). Plasma sCD147 levels were assessed using the EMMPRIN/CD147 ELISA kit (R&D Systems, Minneapolis [MN], US) according to the manufacturer's protocol. The concentration of the sample in each ELISA well was determined by interpolation from a standard curve. Each sample was tested in duplicate.

Statistical analysis

The Mann-Whitney *U* test was used to compare levels of plasma sCD147 in different groups according to variable clinicopathological factors. The Chi squared contingency test with Yates correction was used to determine the relationship between clinicopathological factors of breast cancer patients and lymph node status or chemotherapy sensitivity. Binary logistic regression was used to assess clinicopathological factors (plasma sCD147, tumour size, and *HER2*) that were associated with lymph node metastasis or chemoresistance in invasive breast cancer. All multivariable logistic regression models used backward stepwise procedures, and only datasets complete for every outcome analysed were used. Receiver operating characteristic (ROC) curve analysis was performed to calculate the area under the curve and evaluate the optimal cut-off point, which was given by the maximum of the Youden index. Statistical significance was set at $P<0.05$. The GraphPad Prism 6 software (GraphPad Software, La Jolla [CA], US) and SPSS (Windows version 19.0; IBM Corp, Armonk [NY], US) were used for statistical analysis.

Results

Patient characteristics

Among all eligible patients with complete records, 165 had benign breast disease (age range, 22-68 years) and 308 had primary breast cancer (age range, 24-77 years). There was no significant difference in age between the two groups (P=0.381). Breast cancer patients comprised 11 with ductal carcinoma in situ and 297 with invasive ductal carcinoma. Retrospective analysis of the association of plasma sCD147 level with response to neoadjuvant chemotherapy included 175 patients who met all the inclusion criteria (Fig)—luminal A in 39, luminal B in 70, *HER2*-positive in 28, and TNBC in 38. In all, 170 patients had T2-T4 tumours and five had T1 TNBC tumours. Using the RECIST criteria, we assigned the 175 patients to two groups: chemotherapy-sensitive (n=126) and chemotherapy-resistant (n=49).

Plasma soluble CD147 levels in breast cancer patients

According to ELISA results, plasma sCD147 levels were significantly higher in patients with primary breast cancer than in those with benign breast disease (median [interquartile range; IQR], 8629.81 pg/mL [7426.33-10 309.20 pg/mL] vs 7625.99 pg/mL [6739.20-9140.04 pg/mL]; P=0.001). However, there was no significant difference in plasma sCD147 levels between patients with invasive breast cancer and those with ductal carcinoma in situ (8618.91 pg/mL [7404.81-10 358.50 pg/mL] vs 9185.79 pg/mL [7671.15-9626.47 pg/mL]; P=0.787). Regarding cancer subtypes of the 297 patients with invasive breast carcinoma, median (IQR) plasma sCD147 levels were significantly higher in patients with *HER2*-positive breast cancer (10 042.34 pg/mL [7772.01-11 058.48 pg/mL]) than in those with luminal A tumours (7991.05 pg/mL [7101.72-10 237.4 pg/mL]; P=0.007), luminal B tumours (8629.81 pg/mL [7200.45-9953.32 pg/mL]; P=0.017), and TNBC tumours (8585.16 pg/mL [7884.27-10 545.51 pg/mL]; P=0.027).

Association between plasma soluble CD147 and clinicopathological factors

The association between plasma sCD147 level and clinicopathological factors in patients with invasive breast cancer is summarised in Table 1. Plasma sCD147 levels increased with tumour size: median (IQR) levels were significantly higher in patients with locally advanced (stage T3-T4) than those with early (stage T1-T2) breast cancer (10 093.26 pg/mL [7974.73-11 451.21 pg/mL] vs 8561.45 pg/mL [7169.41-9952.90 pg/mL]; P=0.001). Plasma sCD147 levels were also elevated in patients with lymph node metastasis compared with those without (median [IQR], 9991.42 pg/mL [8154.61-11 452.84 pg/mL] vs 7814.78 pg/mL [6936.82-9516.85 pg/mL]; P<0.001). In addition, plasma sCD147 levels were significantly

TABLE 1. Association between plasma soluble CD147 and various clinicopathological factors (n=297)

	No. of cases	Soluble CD147, pg/mL		P value
		Median	Interquartile range	
Tumour size*				0.001
T1-T2	233	8561.45	7169.41-9952.90	
T3-T4	60	10 093.26	7974.73-11 451.21	
ER status				0.466
Negative	91	8694.41	7631.98-10 545.51	
Positive	206	8586.83	7258.25-10 237.41	
PR status				0.425
<20%	184	8844.08	8443.54-9244.61	
≥20%	113	9249.06	8651.77-9846.34	
Ki67 status				0.518
<14%	136	8178.75	7151.41-10 509.91	
≥14%	161	8629.81	7477.74-10 181.40	
HER2 status				0.160
Negative	208	8568.83	7448.34-10 070.42	
Positive	89	9254.34	7157.63-11 199.38	
LN status				<0.001
Negative	172	7814.78	6936.82-9516.85	
Positive	125	9991.42	8154.61-11 452.84	
Recurrence risk				<0.001
Medium	205	8134.68	7151.41-9616.68	
High	92	10 093.26	8135.35-11 679.71	
Chemotherapy (n=175)				0.040
Sensitive	126	8585.16	7789.74-9868.87	
Resistant	49	10 093.26	7974.73-11 261.88	

Abbreviations: CD147 = cluster of differentiation 147; ER = oestrogen receptor; *HER2* = human epidermal growth factor receptor 2; LN = lymph node; PR = progesterone receptor

* Exact tumour size could not be determined for four patients

higher in patients with a high risk of recurrence than in those with a medium risk (median [IQR], 10 093.26 pg/mL [8135.35-11 679.71 pg/mL] vs 8134.68 pg/mL [7151.41-9616.68 pg/mL]; P<0.001). Although plasma sCD147 levels were elevated for the *HER2*-positive breast cancer subtype as compared with other breast cancer subtypes, there was no significant difference between *HER2*-positive and *HER2*-negative patients (median [IQR], 9254.34 pg/mL [7157.63-11 199.38 pg/mL] vs 8568.83 pg/mL [7448.34-10 070.42 pg/mL]; P=0.160).

Plasma soluble CD147 as an independent predictor for lymph node metastasis

Because plasma sCD147 was associated with lymph node status and recurrent risk, we speculated that plasma sCD147 may be a predictor for lymph node metastasis of breast cancer. Univariate analysis showed that tumour size and *HER2* status may be involved in lymph node metastasis (Table 2). We subsequently used binary logistic regression analysis to identify clinicopathological factors associated with lymph node metastasis in invasive breast cancer. Our data showed that plasma sCD147 (P<0.001), *HER2*-positive tumours (P=0.001), and tumour size T3-T4 (P=0.005) were independent predictors of lymph node metastasis of breast cancer (Table 3). When we analysed ROC curves to evaluate use of plasma sCD147 as a diagnostic biomarker for lymph node metastasis, the area under the curve was 0.745 (95% confidence interval, 0.676-0.813) and the optimal cut-off point of plasma sCD147 was 8577 pg/mL, which provided a sensitivity of 70.9% and a specificity of 61.7%.

TABLE 2. Association between clinicopathological factors and lymph node involvement (n=297)

	Lymph node involvement		χ^2	P value
	Without metastasis	With metastasis		
Tumour size*			18.67	<0.001
T1-T2	153	80		
T3-T4	16	44		
ER status			1.759	0.185
Negative	46	45		
Positive	126	80		
PR status			0.427	0.519
<20%	103	81		
≥20%	69	44		
Ki67 status			1.25	0.266
<14%	79	57		
≥14%	93	68		
HER2 status			12.28	<0.001
Negative	135	73		
Positive	37	52		

Abbreviations: ER = oestrogen receptor; *HER2* = human epidermal growth factor receptor 2; PR = progesterone receptor
* Exact tumour size could not be determined for four patients

Association of plasma soluble CD147 levels with chemotherapy response

Table 1 shows that plasma sCD147 levels in the chemotherapy-resistant group were significantly higher than those in the chemotherapy-sensitive group (median [IQR], 10 093.26 pg/mL [7974.73-11 261.88 pg/mL] vs 8585.16 pg/mL [7789.74-9868.87 pg/mL]; P=0.040). Univariate analysis revealed that tumour size and *HER2* status may be involved in chemotherapy response (Table 4). Binary logistic regression analysis demonstrated that plasma sCD147 was not an independent predictor for chemotherapy response of breast cancer patients, but tumour size of T3-T4 was (P=0.001) [Table 5].

Discussion

The tumour microenvironment plays a proactive role in malignant disease progression, including the transition from ductal carcinoma in situ to invasive cancer, tumour cell proliferation, dissemination, and metastasis.[27] CD147 has been found to be overexpressed in breast cancer, associated with tumour size and staging, and predictive of poor prognosis.[28-31] Tumour cells express molecules, either secreted or presented on the cell surface, that interact with surrounding stromal cells. Soluble CD147 may be released from membrane-associated CD147 as a result of both MMP proteolytic

TABLE 3. Results of multivariable analysis of clinicopathological factors and lymph node metastasis

	B	SE	Wald	P	Exp(B)	95% CI of Exp(B)
Plasma sCD147	2.300	0.499	21.264	<0.001	9.969	3.750-26.504
Tumour size T3-T4	-1.163	0.417	7.767	0.005	0.313	0.138-0.708
HER2 positive	-1.178	0.364	10.443	0.001	0.308	0.151-0.629

Abbreviations: CI = confidence interval; *HER2* = human epidermal growth factor receptor 2; sCD147 = soluble cluster of differentiation 147; SE = standard error

activity and microvesicle shedding in the tumour microenvironment. Soluble CD147 may then act in a paracrine fashion on stromal cells to further trigger production of MMPs and CD147; the latter contributes to tumour angiogenesis, tumour growth, and metastasis.[16,19,21]

Wu et al[15] reported that serum sCD147 enhances the secretion of MMP-2 from hepatocellular carcinoma cells by activating extracellular signal-regulated kinase and focal adhesion kinase, as well as phosphoinositide-3-kinase/Akt signalling, indicating that sCD147 may contribute to hepatocellular carcinoma progression. Moreover, serum sCD147 was elevated in patients with hepatocellular carcinoma compared with healthy individuals, and sCD147 level was associated with tumour size and Child-Pugh score.[15] Gross et al[22] also reported that sCD147 levels were elevated in patients with multiple myeloma, and elevated levels were associated with refractory disease and shortened progression-free survival, indicating that sCD147 may be a new prognostic factor for patients with multiple myeloma.

A previous study demonstrated that CD147 was overexpressed in human breast cancer.[10] In this study, we measured plasma sCD147 levels by ELISA and found that plasma sCD147 levels were significantly elevated in breast cancer patients compared with control patients who had benign breast diseases. We also found that plasma sCD147 was significantly elevated in lymph node metastasis in breast cancer patients. Taken together, these data show that plasma sCD147 may be released from tumour cells and promote lymph node metastasis of breast cancer. Some studies have reported that sCD147 has been detected in patients with inflammatory diseases[31] or cardiovascular diseases.[32,33] To eliminate interference from other diseases and conditions, we excluded patients with inflammatory or cardiovascular diseases and ensured patients in each group had a similar age distribution.

Previous studies have shown that membrane-bound CD147 may correlate with *HER2* expression. Yan et al[34] reported that CD147 induces angiogenesis by stimulating vascular endothelial growth factor production, invasiveness by stimulating MMP production, and multidrug resistance by hyaluronan-mediated upregulation of *HER2* signalling. Xue et al[30] reported that CD147 expression was positively correlated with *HER2* overexpression. In a recent study, CD147 knockdown was shown to improve the antitumour efficacy of trastuzumab in *HER2*-positive breast cancer cells.[35] Although we found that plasma sCD147 levels were significantly higher in the *HER2*-positive breast cancer subtype than in luminal A, luminal B, and TNBC subtypes, plasma sCD147 had no association with expression of *HER2* or oestrogen/progesterone receptors in breast cancer. The reason for this finding is that there are four breast cancer subtypes—luminal A, luminal B, *HER2*-positive, and TNBC—according to oestrogen/progesterone receptor, *HER2*, and Ki67 status. The luminal B subtype includes some breast cancers that are positive for oestrogen/progesterone receptor and *HER2*. Hence, patients who are *HER2*-positive (Table 1) include those with *HER2*-positive subtype

TABLE 4. Association between tumour characteristics and chemotherapy response (n=175)

	Chemotherapy response		χ^2	P value
	Sensitive	Resistance		
Tumour size			19.51	<0.001
T1-T2	104	15		
T3-T4	22	34		
ER status			0.071	0.792
Negative	46	20		
Positive	80	29		
PR status			0.007	0.935
<20%	84	33		
≥20%	42	16		
Ki67 status			1.982	0.169
<14%	73	20		
≥14%	53	29		
HER2 status			8.316	0.004
Negative	85	15		
Positive	41	34		

Abbreviations: ER = oestrogen receptor; *HER2* = human epidermal growth factor receptor 2; PR = progesterone receptor

TABLE 5. Results of multivariable analysis of clinicopathological factors and chemotherapy resistance

	B	SE	Wald	P value	Exp(B)	95% CI of Exp(B)
Plasma sCD147	0.684	0.782	0.766	0.381	1.982	0.428-9.171
Tumour size T3-T4	-2.086	0.649	10.329	0.001	0.124	0.035-0.443
HER2 positive	-0.375	0.670	0.312	0.576	0.688	0.185-2.559

Abbreviations: CI = confidence interval; *HER2* = human epidermal growth factor receptor 2; sCD147 = soluble cluster of differentiation 147; SE = standard error

and also luminal B subtype; plasma sCD147 levels in patients who were '*HER2*-positive' were different from those with a *HER2*-positive subtype.

It is essential to establish predictive factors to allow evaluation of the recurrence risk of breast cancer, so that optimal adjuvant therapy can be selected for individual patients.[3,36] Larger tumour size at diagnosis, high proliferation factors, absence of oestrogen/progesterone receptors and *HER2* overexpression, and lymph node metastasis are related to a high risk of recurrence and poor survival, and are commonly recognised as prognostic and predictive factors for breast cancer recurrence risk.[4,37,38] Consistent with these findings, we found that plasma sCD147 levels were significantly increased in patients with locally advanced lymph node metastasis and a high risk of breast cancer recurrence. We also found that plasma sCD147 was positively associated with tumour size, lymph node metastasis, and high recurrence risk of invasive breast cancer.

Lymph node status, which confers different strategies for patients at different tumour stages, is critical information for the treatment of breast cancer, and the accurate prediction of lymph node status is a prerequisite for treatment decision. Our binary logistic regression analysis showed that plasma sCD147, *HER2* positive subtype, and tumour size (T3-T4) were independent predictors for lymph node metastasis of breast cancer patients. Taken together, these data suggest that plasma sCD147 may be a new factor for the evaluation of breast cancer recurrence risk. Our ROC analysis demonstrated that plasma sCD147 could be a biomarker for distinguishing breast cancer patients with lymph node metastasis from those without; however, the sensitivity and specificity were not high (70.9% and 61.7%, respectively). The relatively low sensitivity and specificity suggest that using plasma sCD147 as the sole biomarker may result in substantial numbers of false positives and false negatives. Therefore, it may be necessary to investigate whether the combination of plasma sCD147 and other biomarkers can improve efficacy.

According to the data of 303 patients who were followed up for 3 to 38 months (median, 20 months), 11 patients had relapse: two had local recurrences and nine had distant metastases. The mean time of recurrence/metastasis was 23.6 months, with no difference between patients with relapse and those without (Table 6). We were not able to investigate the relationship between plasma sCD147 and disease-free survival or overall survival, because of the short median follow-up period.

Previous data have shown that CD147 is one of the apoptosis-related proteins and it may mediate adriamycin chemoresistance in breast cancer by affecting the cellular localisation and dimerisation of the protein ABCG2 (ATP-binding cassette subfamily G member 2).[10] In this study, we studied the relationship between plasma sCD147 and chemotherapy response in invasive breast cancer. All patients were given four cycles of AC/EC-based chemotherapy. We also found that plasma sCD147 levels were significantly higher in the chemotherapy-resistant group than in the chemotherapy-sensitive group, and such levels were positively associated with chemotherapy resistance. Although our data also showed that plasma sCD147, tumour size (T3-T4), and *HER2* positive subtype may be involved in chemotherapy response, binary logistic regression demonstrated that tumour size (T3-T4) was an independent predictor for chemotherapy response of breast cancer patients, but plasma sCD147 was not. Owing to the small number of cases in the chemotherapy-resistant group, the statistical analysis of data may be underpowered.

In addition to the small sample of study and short median follow-up period, there were other limitations in this study. This study was conducted in one centre, and the researchers who extracted the data and conducted the analysis were not blinded to the study hypothesis. There may have increased selection and information bias. Furthermore, as the design of this study was relatively simple, there may be insufficient control for potential confounding factors in the multivariable analysis.

In conclusion, our study found that plasma sCD147 levels were elevated in breast cancer patients compared with controls with benign breast disease, and plasma sCD147 level was associated with tumour size, lymph node metastasis, high recurrence risk, and AC/EC-based chemoresistance. Moreover, our study supports that plasma sCD147 is an independent predictive factor for lymph node metastasis and is a feasible marker to distinguish

TABLE 6. Follow-up data on relapse status

	No. of cases	Soluble cluster of differentiation 147, pg/mL	
		95% Confidence interval	Median
Relapse (locoregional ± distant)	11	7138.11-11 667.90	8399.09
Distant relapse	9	7485.45-12 641.60	9313.10
Relapse-free	292	8692.12-9535.46	8799.68

breast cancer patients with lymph node metastasis from patients without.

Author contributions

Concept or design: LL Tang, LQ Liao.
Acquisition of data: YJ Liu, YH Kuang, SM Wang, GJ Yan.
Analysis or interpretation of data: LL Tang, LQ Liao.
Drafting of the article: YH Kuang, LQ Liao.
Critical revision for important intellectual content: YH Kuang, LQ Liao.
YH Kuang, YJ Liu, and LL Tang contributed equally to this study.

Funding/support

This study was supported by two grants from the National Natural Science Foundation of China (No. 81101654, awarded to LQ Liao, and No. 81573049, awarded to YH Kuang).

Declaration

The authors have no conflicts of interest to disclose. All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Ethical approval

The research protocols for the use of human tissue were approved by and conducted in accordance with the policies of the Institutional Review Boards at Central South University (Ref No. 201403152), which were formulated based on the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all participants.

References

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
2. Rivenbark AG, O'Connor SM, Coleman WB. Molecular and cellular heterogeneity in breast cancer: challenges for personalized medicine. Am J Pathol 2013;183:1113-24.
3. Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 2011;22:1736-47.
4. Soerjomataram I, Louwman MW, Ribot JG, et al. An overview of prognostic factors for long-term survivors of breast cancer. Breast Cancer Res Treat 2008;107:309-30.
5. Adaniel C, Jhaveri K, Heguy A, et al. Genome-based risk prediction for early stage breast cancer. Oncologist 2014;19:1019-27.
6. Weigelt B, Peterse JL, van 't Veer LJ. Breast cancer metastasis: markers and models. Nat Rev Cancer 2005;5:591-602.
7. Redig AJ, McAllister SS. Breast cancer as a systemic disease: a view of metastasis. J Intern Med 2013;274:113-26.
8. O'Shaughnessy J. Extending survival with chemotherapy in metastatic breast cancer. Oncologist 2005;10 Suppl 3:20-9.
9. Kuang YH, Chen X, Su J, et al. RNA interference targeting the CD147 induces apoptosis of multi-drug resistant cancer cells related to XIAP depletion. Cancer Lett 2009;276:189-95.
10. Zhou S, Liao L, Chen C, et al. CD147 mediates chemoresistance in breast cancer via ABCG2 by affecting its cellular localization and dimerization. Cancer Lett 2013;337:285-92.
11. Yang JM, Xu Z, Wu H, et al. Overexpression of extracellular matrix metalloproteinase inducer in multidrug resistant cancer cells. Mol Cancer Res 2003;1:420-7.
12. Marieb EA, Zoltan-Jones A, Li R, et al. Emmprin promotes anchorage-independent growth in human mammary carcinoma cells by stimulating hyaluronan production. Cancer Res 2004;64:1229-32.
13. Nabeshima K, Iwasaki H, Koga K, et al. Emmprin (basigin/CD147): matrix metalloproteinase modulator and multifunctional cell recognition molecule that plays a critical role in cancer progression. Pathol Int 2006;56:359-67.
14. Zhao S, Ma W, Zhang M, et al. High expression of CD147 and MMP-9 is correlated with poor prognosis of triple-negative breast cancer (TNBC) patients. Med Oncol 2013;30:335.
15. Wu J, Hao ZW, Zhao YX, et al. Full-length soluble CD147 promotes MMP-2 expression and is a potential serological marker in detection of hepatocellular carcinoma. J Transl Med 2014;12:190.
16. Tang Y, Kesavan P, Nakada MT, et al. Tumor-stroma interaction: positive feedback regulation of extracellular matrix metalloproteinase inducer (EMMPRIN) expression and matrix metalloproteinase-dependent generation of soluble EMMPRIN. Mol Cancer Res 2004;2:73-80.
17. Bordador LC, Li X, Toole B, et al. Expression of emmprin by oral squamous cell carcinoma. Int J Cancer 2000;85:347-52.
18. Taylor PM, Woodfield RJ, Hodgkin MN, et al. Breast cancer cell-derived EMMPRIN stimulates fibroblast MMP2 release through a phospholipase A(2) and 5-lipoxygenase catalyzed pathway. Oncogene 2002;21:5765-72.
19. Sidhu SS, Mengistab AT, Tauscher AN, et al. The microvesicle as a vehicle for EMMPRIN in tumor-stromal interactions. Oncogene 2004;23:956-63.
20. Egawa N, Koshikawa N, Tomari T, et al. Membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14) cleaves and releases a 22-kDa extracellular matrix metalloproteinase inducer (EMMPRIN) fragment from tumor cells. J Biol Chem 2006;281:37576-85.
21. Hanata K, Yamaguchi N, Yoshikawa K, et al. Soluble EMMPRIN (extra-cellular matrix metalloproteinase inducer) stimulates the migration of HEp-2 human laryngeal carcinoma cells, accompanied by increased MMP-2 production in fibroblasts. Arch Histol Cytol 2007;70:267-77.
22. Gross Z, Udd K, Ghermezi M, et al. Serum CD147 levels are increased in multiple myeloma patients and elevated levels are associated with refractory disease and shortened progression free survival. Am Soc Hematology 2016;128:5652.
23. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 2005;97:1180-4.
24. Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013;24:2206-23.

25. Goldhirsch A, Wood WC, Gelber RD, et al. Progress and promise: highlights of the international expert consensus on the primary therapy of early breast cancer 2007. Ann Oncol 2007;18:1133-44.
26. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
27. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. Nature 2001;411:375-9.
28. Dalberg K, Eriksson E, Enberg U, et al. Gelatinase A, membrane type 1 matrix metalloproteinase, and extracellular matrix metalloproteinase inducer mRNA expression: correlation with invasive growth of breast cancer. World J Surg 2000;24:334-40.
29. Reimers N, Zafrakas K, Assmann V, et al. Expression of extracellular matrix metalloproteases inducer on micrometastatic and primary mammary carcinoma cells. Clin Cancer Res 2004;10:3422-8.
30. Xue S, Li SX, Wu ZS, et al. Expression of CD147, matrix metalloproteinases and transforming growth factor beta1 in breast cancer [in Chinese]. Zhonghua Bing Li Xue Za Zhi 2009;38:524-8.
31. Yanaba K, Asano Y, Tada Y, et al. Increased serum soluble CD147 levels in patients with systemic sclerosis: association with scleroderma renal crisis. Clin Rheumatol 2012;31:835-9.
32. Major TC, Liang L, Lu X, et al. Extracellular matrix metalloproteinase inducer (EMMPRIN) is induced upon monocyte differentiation and is expressed in human atheroma. Arterioscler Thromb Vasc Biol 2002;22:1200-7.
33. Schmidt R, Bultmann A, Fischel S, et al. Extracellular matrix metalloproteinase inducer (CD147) is a novel receptor on platelets, activates platelets, and augments nuclear factor kappaB-dependent inflammation in monocytes. Circ Res 2008;102:302-9.
34. Yan L, Zucker S, Toole BP. Roles of the multifunctional glycoprotein, emmprin (basigin; CD147), in tumour progression. Thromb Haemost 2005;93:199-204.
35. Xiong L, Ding L, Ning H, et al. CD147 knockdown improves the antitumor efficacy of trastuzumab in *HER2*-positive breast cancer cells. Oncotarget 2016;7:57737-51.
36. Cianfrocca M, Goldstein LJ. Prognostic and predictive factors in early-stage breast cancer. Oncologist 2004;9:606-16.
37. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287-312.
38. Taneja P, Maglic D, Kai F, et al. Classical and novel prognostic markers for breast cancer and their clinical significance. Clin Med Insights Oncol 2010;4:15-34.