Correlation between connexin 43 expression in circulating tumor cells and biological characteristics of breast cancer

Dan-Qing Wang [a], Yuan-Yuan Wang [b], Yan-Ling Shi [a], Bin Zeng [a], Zi-Jing Lin [a], Qin Deng [a,**], Jia Ming [a,*]

[a] *Department of Breast and Thyroid Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, People's Republic of China*
[b] *Department of Emergency, Xi'an Central Hospital, Xi'an 710003, People's Republic of China*

A R T I C L E   I N F O

A B S T R A C T

Background: Connexin 43 (Cx43) has been closely linked to the occurrence and progression of breast cancer. Distant metastasis of breast cancer is aided by the epithelial-mesenchymal transition of circulating tumor cells (CTCs). However, the impact of Cx43 expression on CTCs and the extent of its role in the disease remain unclear.
Methods: We determined CTCs in 156 patients, who had breast cancer with a disease course of two or more years. We also measured the expression of Cx43 in the CTCs. The CTCs were detected in the blood of 139 of these patients. These 139 patients were divided into two groups: the Cx43 group and the non-Cx43 group based on their Cx43 expression.
Results: Overall, Cx43 expression was found in 83 of the 139 patients (59.7%, 83/139 cases). The two groups significantly differed in terms of the number of mixed biphenotypic type CTCs and the total number of CTCs ($P < 0.05$). There were significant correlations between Cx43 expression and Ki67 expression, tumor size, lymph node metastasis, and TNM stage ($P < 0.05$ for all). The data suggested that patients with Cx43 expression had a higher risk of distant metastasis and had later-stage disease. The difference in Cx43 expression between patients with and without epidermal growth factor receptor 2 (Her2) overexpression was statistically significant ($P < 0.05$). The difference in disease-free survival (DFS) between the two groups was statistically significant ($P = 0.03$), and the Cx43 group had a shorter duration of DFS. Univariate Cox regression analysis revealed that Cx43 expression, Her2 expression, and tumor size were significantly correlated with DFS ($P = 0.03$, 0.0023, and 0.01, respectively).
Conclusion: Cx43 expression in the CTCs of patients with breast cancer is a cancer-promoting factor.

1. Introduction

At present, breast cancer is the malignant tumor with the highest incidence. There were 2.2 million new cases of breast cancer worldwide in 2020 [1]. The incidence is increasing year by year while the average age of those affected is reducing [2]. Around 11% of the world's breast cancer occurs in China, significantly impacting the health of women [3]. The China National Cancer Control Center (2017) estimates the following total five-year survival rates of patients with breast cancer in China: 96.5% in clinical stage I, 91.6% in clinical stage II, 74.8% in clinical stage III, and 40.7% in clinical stage IV [4]. Moreover, distant metastases to the liver, lungs, bones, and brain are common in breast cancer. Once distant metastasis occurs, it often worsens the prognosis. Therefore, it is particularly important to assess the risk of recurrence and metastasis during the early stages of breast cancer.

Circulating tumor cells (CTCs) are tumor cells that break off and metastasize into the peripheral blood system; they exist in the blood in the form of epithelial cells, mixed biphenotypic type cells, and mesenchymal cells and are an important research index of malignant tumor blood metastasis. At present, CTCs are primarily used to assess the prognosis of malignant tumors [5,6]. Furthermore, they can provide useful insights on tumorigenesis and tumor evolution. A previous study found that a high CTC count ($\geq$5 CTCs/7.5 ml) is an important adverse prognostic factor affecting overall survival (OS) and disease-free survival (DFS) [7]. Thus, CTCs have the potential to be used in the prediction of DFS and overall survival among patients with early breast cancer.

In pre-experiments, we found that connexin 43 (Cx43) is expressed on CTCs. Connexins are a family of transmembrane proteins that play a transmission role in cell communication [8–10] by forming gap junction intercellular channels between cells and directly connecting the cytoplasm of adjacent cells. Each gap junction consists of a hexagonal half channel arranged on the cell membrane of two adjacent cells and composed of two hexamer connexin oligomers [11]. Connexin 43 is one of the most highly expressed and widely studied connexins. It has been found to be abnormally expressed in several tumor types, including liver, prostate, and mammary gland tumors [12–15]. It impacts cell adhesion, the interaction between tumor cells and the cell microenvironment, and metastasis potential [16], thus playing a role in the occurrence and development of breast cancer and the metastasis of tumor cells. However, it is not clear whether Cx43 is a cancer-promoting factor or acts as a tumor suppressor.
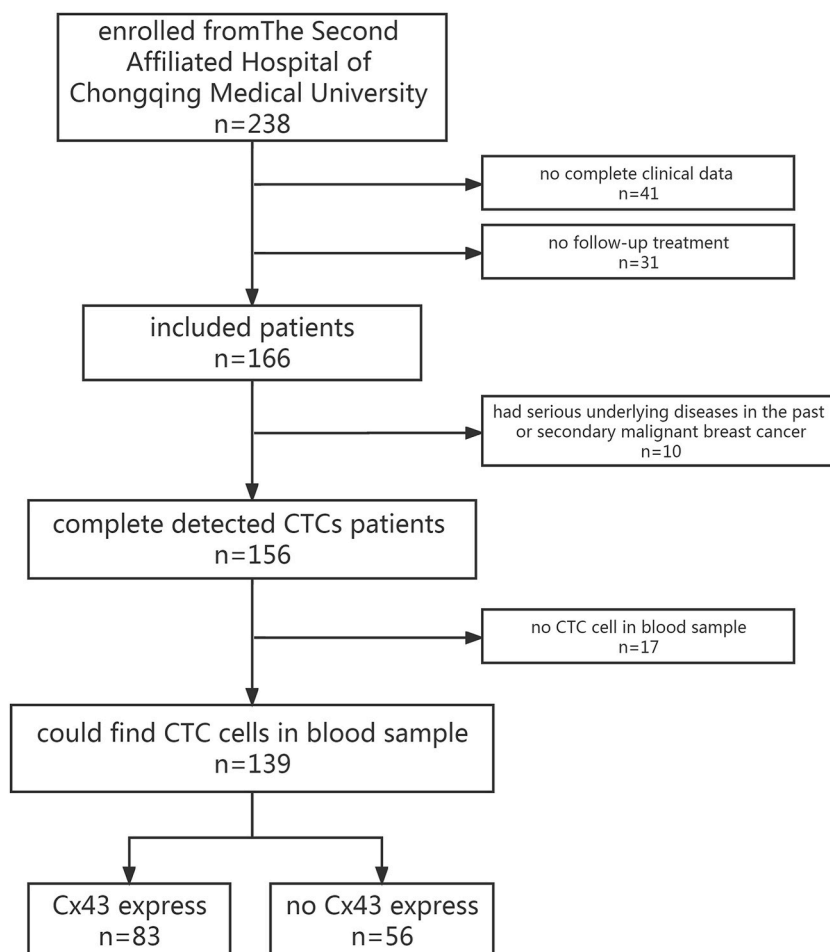

**Fig. 1.** Flow chart of patient inclusion and grouping.

At present, most relevant studies on Cx43 focus on breast tissue and distant metastatic lesions, while the expression of Cx43 in CTCs has not been reported. The biological significance of this study is that Cx43, in its role as a connexin, exists between cells and is an important signal pathway in breast cancer. Therefore, we detected Cx43 in the CTC of patients with breast cancer and evaluated its expression. CTC is a necessary condition for tumor metastasis. Based on this finding, we studied the expression of Cx43 in the CTC of breast cancer patients and identified the role of Cx43 in it to provide new information for understanding the distant metastasis of breast cancer.

2. Materials and methods

We obtained the approval of the Ethics Committee of The Second Affiliated Hospital of Chongqing Medical University (2022–19) for this study and conducted it in compliance with all regulations as per the Declaration of Helsinki. All participants provided their written informed consent.

2.1 Inclusion criteria: 1. Patients diagnosed with breast cancer in our hospital; 2. Patients aged 18–75 years old; 3. Patients receiving standard comprehensive treatment (chemotherapy, targeted therapy, endocrine therapy, radiotherapy, and surgery) in our center; 4. Pathology diagnosis confirmed to be breast cancer; 5. Patients completed treatment and received regular follow-up in our center; and 6. The duration of the disease was more than two years.

Exclusion criteria: 1. Patients who had serious underlying diseases in the past (e.g., advanced non-breast primary malignant tumors or serious cardiovascular, cerebrovascular, renal, or respiratory diseases); 2. Those with missing clinical data; 3. Patients lost to follow-up; and 4. Patients with secondary malignant breast cancer.
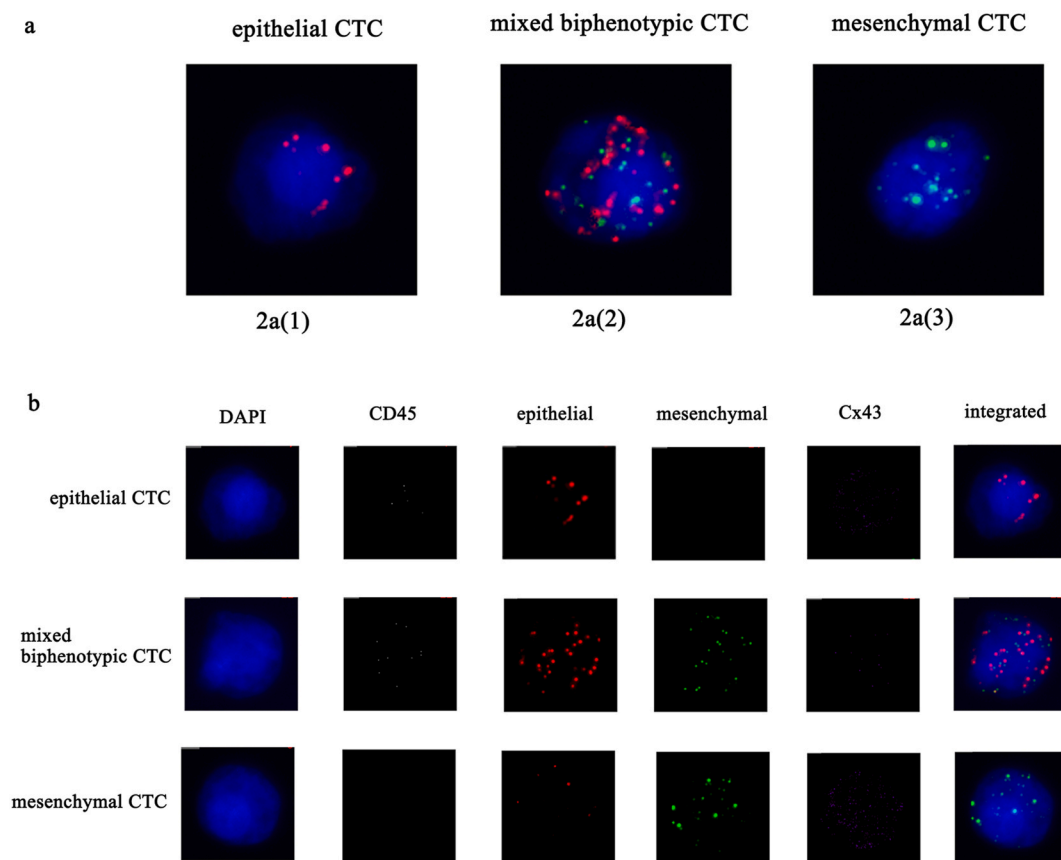
Fig. 2. CTCs detected in blood samples from patients with breast cancer.
2a:CTC phenotypes classified using epithelial and mesenchymal markers. Epithelial CTCs (2a (1)) stained with red dots only; mixed biphenotypic CTCs (2a (2)) stained with both red and green dots; mesenchymal CTCs (2a (3)) stained with green dots only.
2 b:Cx43 expression in CTCs. Representative images based on mRNA-ISH of epithelial (red dots), mesenchymal (green dots), CD45 (bright white dots), and Cx43 (purple dots) markers. Epithelial CTCs expressing Cx43 (first line) exhibit red and purple dots; mixed biphenotypic CTCs expressing Cx43 (second line) exhibit red, green, and purple dots; mesenchymal CTCs expressing Cx43 (third line) exhibit green and purple dots.

2.1. Experimental grouping

In this study, we detected the expression of CTCs and Cx43 in 156 cases of breast cancer, and CTCs were detected in 139 of the patients. These 139 patients with CTCs were divided into two groups based on the expression of Cx43, i.e., the Cx43 group and the non-Cx43 group (Fig. 1).

2.2. Basic patient information

We collected the following details of each patient: age, menstrual status at the time of tumor onset, pathological features of breast cancer (including histological grade, tumor type, and tumor size), lymph node metastasis, distant metastasis, tumor stage, distant metastasis-free time (from the initial diagnosis of breast cancer to the month when the tumor progressed and distant metastasis occurred), and CTC hematological results (i.e., the total CTC number, cell type, number of various cell types, and expression of Cx43 in each cell type).

2.3. CTC collection and Cx43 detection

2.3.1. CTC collection

We collected 7 ml of peripheral blood in an ethylenediamine tetraacetic acid tube from each patient. The samples were transferred to sample preservation tubes containing erythrocyte lysis buffer using a customized connection device, and the erythrocytes were lysed for 30 min at room temperature. After centrifuging the resulting lysates for 5 min at 600 g, the cell pellets were resuspended in phosphate-buffered saline containing 4% formaldehyde for 8 min. Finally, the CTCs were isolated by filtration through calibrated membranes with 8 μm pores.
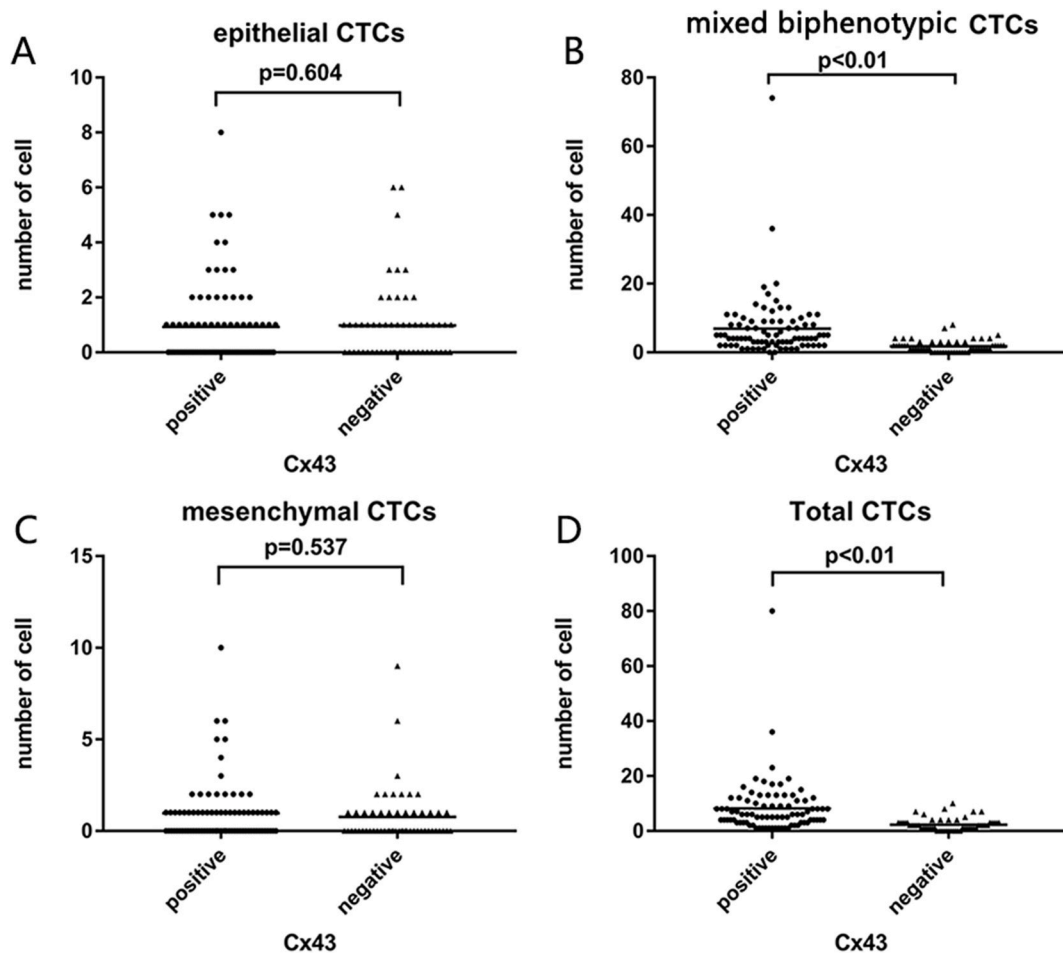
Fig. 3. Differences in CTC types between the Cx43 and non-Cx43 groups. Differences in (A) epithelial CTCs. (B) Mixed biphenotypic CTCs. (C) Mesenchymal CTCs. (D) Total CTCs between the Cx43 and non-Cx43 groups.

2.3.2. Identification and classification of CTCs and detection of Cx43

A multiplex mRNA in situ hybridization (ISH) assay was used to identify and classify CTCs. The ISH test of mRNA was performed on a 24-well plate. The enriched cells on the plate were treated with protease and then hybridized with epithelial biomarkers EpCAM and CK8/18/19, mesenchymal biomarkers Vimentin and Twist, leukocyte biomarker CD45, and gene probe-labeled Cx43. Hybridization was carried out at 42 °C for 2 h; then, the cells were washed using 1000 μL of washing buffer. The sample was mixed with 100 μL of signal pre-amplification solution and placed at 42 °C for 20 min. The nanofilm was cooled and rinsed with 1000 μL of washing buffer. Four types of fluorescently labeled probes were added to couple with the fluorescent dyes Alexa Fluor 594 (detecting epithelial biomarkers EpCAM and CK8/18/19), Alexa Fluor 488 (detecting interstitial biomarkers Vimentin and Twist), Alexa Fluor 647 (detecting leukocyte biomarkers), and cy53 (detecting Cx43 gene probe markers) and incubated at 42 °C for 20 min. The above tests were completed by SurExam Bio-Tech Co., Ltd., China. The ISH results were analyzed by qualified pathologists using an automated fluorescence microscope. If the number of fluorescent dots for the epithelial and mesenchymal markers or for CD45 was ≥ 7, they were considered to be valid fluorescent signals. Red and green fluorescent signals indicated the expression of epithelial and mesenchymal markers, respectively, whereas bright white fluorescent signals indicated CD45 expression. A purple fluorescent signal from the CTCs indicated Cx43 expression. The expression of Cx43 in the CTCs is presented in Fig. 2.

2.4. Statistical analysis

The statistical analysis was conducted using the SPSS 24.0 software (SPSS, U.S.A.). The Chi-square analysis was used for the univariate analysis, and the log-rank test and logistic regression were employed for the multivariate analysis. The survival curve was analyzed using the Kaplan-Meier curve. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Differences in CTC type, number, and Cx43 expression

In this study, we collected blood samples from 156 patients with breast cancer, and we found CTCs in the blood samples of 139 of these patients. Among these 139 patients, we determined the expression of Cx43 in the CTCs in 83 patients. Cx43 was expressed in epithelial, mixed bi-phenotypic, and mesenchymal CTCs. Then, we compared the CTCs between the Cx43 and non-Cx43 groups and found that the differences between these groups in the number of mixed biphenotypic type CTCs and the total number of CTCs were statistically significant ($P < 0.05$, Fig. 3B and D). However, the differences in the number of epithelial CTCs ($P = 0.604$, Fig. 3A) and mesenchymal CTCs ($P = 0.537$, Fig. 3C) were not statistically significant.

The median number of mixed biphenotypic type CTCs in the Cx43 group was 4, which was higher than that in the non-Cx43 group (1), and the median total number of CTCs in the two groups was 6 and 2, respectively. We also found that there were similar differences in the number of patients with a large mixed biphenotypic type CTC count and total CTC count between the groups (both $P < 0.01$, Table 1); the differences in the number of epithelial CTCs ($P = 0.887$, Table 1) and mesenchymal CTCs ($P = 0.517$, Table 1) were not statistically significant. The Cx43 group had greater numbers of mixed biphenotypic type CTCs and the total number of CTCs, and there were more patients with high CTC numbers in this group.

3.2. Analysis of clinical characteristics of patients

In this study, we analyzed the data of 139 patients with CTCs detected in their blood. Their median age was 54 years (31–75 years old). The detailed characteristics of patients are listed in Table 2. Among them, we found Cx43 expressed in the CTCs in 83 patients (59.7%, 83/139 cases) but not in the remaining 56 patients (40.2%, 56/139 cases). The expression of Cx43 was significantly correlated with the expression of Ki67, tumor size, lymph node metastasis, and TNM stage ($P < 0.05$ for all, Table 2).

Table 1
Differences in three types of CTCs between the Cx43 group and non Cx43 group.

	Total number (%)	Cx43 group (%)	Non Cx43 group (%)	P
Total number of patients	139 (100.0)	83 (59.7)	56 (40.3)	
Number of epithelial CTCs				
<5	132 (95.0)	79 (56.8)	53 (38.1)	0.887
≥5	7 (5.0)	4 (2.9)	3 (2.2)	
Number of mixed CTCs				
<5	96 (69.1)	43 (30.9)	53 (38.1)	0.000
≥5	43 (30.9)	40 (2.88)	3 (2.2)	
Number of mesenchymal CTCs				
<5	132 (95.0)	78 (56.1)	54 (38.8)	0.517
≥5	7 (5.0)	5 (3.6)	2 (1.4)	
Total number of CTCs				
<5	80 (57.6)	32 (23.0)	48 (34.5)	0.000
≥5	59 (42.4)	51 (36.7)	8 (5.8)	

*The population with high CTC count is: CTCs $\geq 5/7.5$ ml.

Table 2
Relationship between CX43 expression and clinical characteristics in 139 breast cancer patients undergoing CTC detection.

Clinical variable	Total number (%)	Cx43 group (%)	Non Cx43 group (%)	P
All patients	139 (100.0)	83 (59.7)	56 (40.3)	
Age (years old)				
≥50	93 (66.9)	53 (38.1)	40 (28.8)	0.352
<50	46 (33.1)	30 (21.6)	16 (11.5)	
Tumor size				
≥2 cm	96 (69.1)	63 (45.3)	33 (23.7)	0.034
<2 cm	43 (30.9)	20 (14.4)	23 (16.5)	
Ki67				
High expression	107 (77.0)	69 (49.6)	38 (27.3)	0.036
Low expression	32 (23.0)	14 (10.1)	18 (12.9)	
Lymph node metastasis				
Y	55 (39.6)	38 (27.3)	16 (11.5)	0.041
N	84 (60.4)	45 (32.4)	40 (28.8)	
Distant metastasis				
Y	32 (23.0)	22 (15.8)	10 (7.2)	0.235
N	107 (77.0)	61 (43.9)	46 (33.1)	
Molecular typing				
Luminal A type	42 (30.2)	23 (16.5)	19 (13.7)	0.08
Luminal B type	44 (31.7)	25 (18.0)	19 (13.7)	
Her-2 overexpression type	23 (16.5)	19 (13.7)	4 (2.9)	
Triple-negative type	30 (21.6)	16 (11.5)	14 (10.1)	
TNM stage				
Stage I	35 (25.2)	12 (8.6)	23 (16.5)	0.006
Stage II	56 (40.3)	38 (27.3)	18 (12.9)	
Stage III	16 (11.5)	11 (7.9)	5 (3.6)	
Stage IV	32 (23.0)	22 (15.8)	10 (7.2)	
Whether menopause				
Y	89 (64.0)	53 (38.1)	36 (25.9)	0.959
N	50 (36.0)	30 (21.6)	20 (14.4)	
Pathological type				
Special type	13 (9.4)	6 (4.3)	7 (5.0)	0.295
Non special type	96 (69.1)	77 (55.4)	49 (35.3)	

The Cx43 group had more patients with high Ki67 expression, a tumor >2 cm, and lymph node metastasis when compared with the non-Cx43 group. The results were as follows: The number of patients with a tumor >2 cm, a higher expression of Ki67, and lymph node metastasis was 63 (75.9%, 63/83 cases), 69 (83.1%, 69/83 cases), and 38 (45.8%, 38/83 cases) in the Cx43 group, respectively, and 33 (58.9%, 33/56 cases), 38 (67.9%, 38/56 cases), and 16 (28.6%, 16/56 cases) in the non-Cx43 group, respectively. The differences between the groups were statistically significant ($P < 0.05$ for all, Table 2).

However, Cx43 expression was not correlated with age, menstrual status, distant metastasis, overall molecular type, or type of pathology ($P > 0.05$ for all, Table 2). In this study, the TNM stage results indicated that the number of patients with Cx43 expression accounted for 34.2% of the total number of stage I patients, while such patients accounted for 67.8%, 68.7%, and 68.7% of those with stages II, III, and IV, respectively. The number of patients without Cx43 expression accounted for 65.8%, 32.2%, 31.3%, and 31.3% in those with stages I, II, III, and IV, respectively. It can be seen that as the stage advanced, the proportion of patients with Cx43 expression showed an upward trend, suggesting that such patients tended to be diagnosed at a more advanced stage (Table 3).

3.3. Relationship between Cx43 and genotype

Although there was no significant difference in the overall molecular type between the Cx43 group and the non-Cx43 group (P = 0.08, Table 2), when we further compared the Cx43 expression in terms of molecular type, we found a statistically significant difference in Cx43 expression between the patients with and without epidermal growth factor receptor 2 (Her2) overexpression ($P < 0.05$, Table 4). Among the 23 patients with Her2 overexpression, Cx43 expression in the CTCs was detected in 19 of them (82.6%, 19/23 cases) and not detected in only 4 (17.4%, 4/23 cases). In other words, Cx43 was expressed more frequently in patients with HER2

Table 3
Proportion of patients with Cx43 expression and CTC in each stage.

	Stage I	Stage II	Stage III	Stage IV
Total number of patients	35	56	16	32
Number of patients with Cx43 expression	12	38	11	22
Number of patients without Cx43 expression	23	18	5	10
Proportion of patients with Cx43 expression	34.2%	67.8%	68.7%	68.7%
Median number of mixed cells	1	3	3.5	4
Median total number of CTCs	4	4	5.5	4.5

Table 4
Expression of various molecular types between the Cx43 and non Cx43 groups.

Molecular type	Total number (%)	Cx43 group (%)	Non Cx43 group (%)	P
	139 (100.0)	83 (59.7)	56 (40.3)	
Luminal A type				
Y	42 (30.2)	23 (16.5)	19 (13.7)	0.434
N	97 (69.8)	60 (43.2)	37 (26.6)	
Luminal B type				
Y	44 (31.7)	25 (18.0)	19 (13.7)	0.636
N	95 (68.3)	58 (41.7)	37 (26.6)	
Her-2 overexpression type				
Y	23 (16.5)	19 (13.7)	4 (2.9)	0.014
N	116 (83.5)	64 (46.0)	52 (37.4)	
Triple-negative type				
Y	30 (21.6)	16 (11.5)	14 (10.1)	0.421
N	109 (78.4)	67 (48.2)	42 (30.2)	

overexpression. The proportion of patients with Cx43 expression was 54.8% (23/42 cases), 56.8% (25/44 cases), and 53.3% (16/30 cases) in luminal type A, luminal type B, and triple-negative patients, respectively. There was no significant difference in Cx43 expression among the three genotypes ($P > 0.05$, Table 4).

3.4. Cx43 and prognosis of patients with breast cancer

Among the patients with Cx43 expression, 38 (45.7%, 38/83 cases) had lymph node metastasis, and 22 (26.5%, 22/83 cases) had distant metastasis (to bone, lungs, or brain) within two years. In the 56 patients without Cx43 expression, the number of those with lymph node metastasis and distant metastasis within two years was 16 (28.6%, 16/56 cases) and 10 (17.8%, 10/56 cases), respectively. Lymph node metastasis varied significantly between the two groups ($P = 0.041$, Table 2). Patients with Cx43 expression were more prone to lymph node metastasis. Although the statistics revealed that there was no significant difference in distant metastasis between the groups ($P > 0.05$, Table 2), it was observed that patients with Cx43 expression were more prone to distant metastasis. This also suggests that expression of Cx43 leads to a poor prognosis.

Among the 139 patients who underwent the disease evaluation follow-up, the duration of follow-up ranged from 24 to 85 months (median: 35 months). We analyzed the DFS of patients in both the Cx43 group and the non-Cx43 group and found that the difference in the DFS between the groups was statistically significant ($P = 0.03$, Fig. 4); patients in the Cx43 group had a shorter DFS than the non-Cx43 group.

Univariate Cox regression analysis revealed that Cx43 expression, Her2 expression, and tumor size were significantly correlated with DFS ($P = 0.03$, 0.0023, and 0.01, respectively, Table 5). Using multivariate Cox regression analysis, we found that only tumor size was an independent prognostic factor for DFS ($P = 0.002$, Table 5).

4. Discussion

Breast cancer is the most common malignancy, with the highest incidence in women. Approximately 20%–30% of patients with early breast cancer experience a recurrence in 1–2 years after the initial diagnosis, and patients with negative hormone receptors relapse even earlier [17,18]. It is estimated that 10%–15% of patients with breast cancer develop distant metastasis within three years after diagnosis and that the five-year survival rate of such patients is only 23% [19,20].

Distant metastasis is the leading cause of mortality in patients with cancer; this process is initially facilitated by the release of cancer cells from the primary tumor into the blood. Numerous studies have linked CTCs with the recurrence and distant metastasis of breast cancer. CTCs were detected in the peripheral blood of 40%–80% of patients with breast cancer [21–25]. In the current study, we analyzed blood samples from 156 patients with breast cancer, and CTCs were detected in 139 of them.
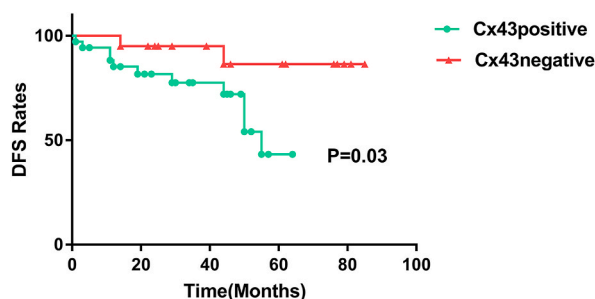
Fig. 4. Kaplan-Meier curves for disease-free progression of the Cx43 group and non-Cx43 group.

Table 5

Univariate and multivariate Cox regression analysis of the relationship between Cx43 expression and patient survival.

DFS	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Tumor size	5.35 (1.18–24.25)	0.01	1.82 (1.25–2.65)	0.002
Her2 expression	5.97 (1.63–21.86)	0.0023	3.79 (0.93–15.49)	0.064
Ki67 expression	1.97 (0.44–8.93)	0.37	1.99 (0.41–9.71)	0.395
Cx43 expression	4.64 (1.01–21.18)	0.03	1.45 (0.26–8.05)	0.675

DFS:disease progression free survival HR:hazard ratio CI:confidence interval.

Circulating tumor cells exist in the blood in many forms, including the epithelial type, mixed biphenotypic type, and mesenchymal type, and are capable of traveling great distances through the circulatory system to implant and metastasize. Distant metastasis can be predicted by detecting tumor cells in the patient's blood. The dissemination of CTCs has been found to correlate with poor prognosis and treatment failure across a variety of tumor types [26]. Further studies have revealed that completely mesenchymal tumor cells can easily enter blood vessels but cannot easily metastasize [27–29]. Tumor cells that are intermediate between the epithelial and mesenchymal types, i.e., mixed biphenotypic type CTCs, can exhibit the characteristics of tumor stem cells. Yu et al. [30] monitored CTCs in 11 patients with breast cancer and found that most of the CTCs were of the mixed biphenotypic type. Different types of CTCs evolve dynamically during treatment. The number of single CTCs can also predict DFS and overall survival in patients with early breast cancer, either before or after breast cancer surgery [31–33]. In the present study, we detected various cell types in the CTCs of patients.

We focused further on the expression of Cx43 in the CTCs of patients in our study. Connexin 43 is a channel protein that plays a role in the occurrence and development of breast cancer. This channel protein has been well researched, and several studies [34–38] have revealed that Cx43 expression is upregulated during the progression of breast cancer and that its expression level is correlated with breast cancer metastasis. It supports the occurrence and progression of breast tumors and the persistence of metastatic lesions [39]. Previous studies [36,39–41] have also revealed that Cx43 expression is upregulated in breast cancer metastasis, suggesting that the protein may play a role in the process of late metastasis. Furthermore, the activity of Cx43 in the metastasis process may not always be inherent in tumor cells. Evidence [42] suggests that Cx43 is significantly expressed in the stroma during the process of cancer progression, suggesting that it may regulate invasion and metastasis through the interactions between epithelial tumor cells and the stroma. Our results in this study are consistent with this.

Previous studies have demonstrated that forced expression of Cx43 in breast cancer cell lines reduces migration and angiogenesis [43–48]. Downregulation of Cx43 or loss of GJIC is associated with increased migration and expression of angiogenic factors. The functional relevance of Cx43 to the location and activity of gap junctions in breast cancer is still unclear, as is the role of Cx43 in breast cancer metastasis. Tumor metastasis is inseparable from CTCs. In the present study, we examined the expression of Cx43 appearing in CTCs, which has not been discussed in previous papers. We found that Cx43 was expressed in all CTC phenotypes. Furthermore, when compared with patients without Cx43 expression, there were larger numbers of mixed biphenotypic type CTCs and total CTCs, as well as a higher proportion of high CTCs among those with Cx43 expression. There were no significant differences in the epithelial and mesenchymal CTCs between the groups.

Mixed biphenotypic type CTCs may be an embodiment of the epithelial-mesenchymal transition process, to some extent. CTCs undergo epithelial-mesenchymal transition, i.e., epithelial cells lose their apical-basal polarity, regulate their cytoskeleton, and show a reduction in adhesion between each other [49]. Cells can acquire mesenchymal characteristics on their own or together and increase their motility and invasiveness. The epithelial–mesenchymal transition is considered an important driver of tumor invasion, metastasis, and dispersion. Epithelial-mesenchymal transition activation provides tumor cells with the abilities to migrate, invade, infiltrate, and exude [50,51]. In other words, the epithelial-mesenchymal transition process indicates that cells have abscission, metastasis, and implantation abilities. Mixed biphenotypic type cells are intermediate products of this process. In a sense, an increase in mixed biphenotypic CTCs means that a tumor is more likely to have distant metastasis. Likewise, the quantitative situation of mixed biphenotypic CTCs is also an embodiment of the epithelial-mesenchymal transition process, to some degree. In this study, we found that Cx43 expression differed between all types of CTCs; its expression was obvious in mixed biphenotypic type cells, suggesting that Cx43 may be involved in CTC phenotype expression.

Additionally, we also analyzed the clinical characteristics of patients in this study; and found that the expression of Cx43 was correlated with the expression of Ki67, tumor size, lymph node metastasis, and TNM stage. Patients with Cx43 expression were more likely to have high levels of Ki67, large tumor sizes, and lymph node metastasis, but this was not correlated with age, menstrual status, distant metastasis, overall molecular type, or type of pathology.

We also found that Cx43 is expressed differently in different genotypes. The well-known molecular typing method for breast cancer was originally proposed at the St. Gallen Conference in 2011. Patients were classified as luminal A type, luminal B type, HER2 overexpression type, and triple negative type based on the expression of estrogen receptor, progesterone receptor, Her2, and Ki67 and the results of fluorescence ISH detection. In the present study, Cx43 expression was higher in the Her2 overexpression population, suggesting that Her2 may be somewhat related to Cx43. There was no significant difference in Cx43 expression among the other three genotypes. However, there has been no relevant research on the relationship between HER2 and Cx43. As a result, we boldly speculate that the *C-erbB-2* gene may promote the expression of Cx43. Of course, this needs to be verified with relevant basic experiments.

More importantly, we examined the prognosis of patients in the current study. Our analysis revealed that patients with breast cancer with Cx43 expression in CTCs were in a later TNM stage than patients without Cx43 expression, and former patients were more

likely to develop lymph node metastasis and distant metastasis. When we analyzed the DFS of the two groups, we found that the Cx43 group had a shorter DFS than the non-Cx43 group, which suggests that Cx43 is involved in the metastasis process. As a result, assessment of Cx43 expression in CTCs could be relevant for the evaluation of metastasis in patients with breast cancer.

Univariate Cox regression analysis revealed that Cx43 expression, Her2 expression, and tumor size were correlated with DFS. However, the multivariate Cox regression analysis revealed that only tumor size was an independent prognostic factor for DFS. This might be because our sample size was not large enough, which caused the correlation between Cx43 expression and DFS to be found in the Cox univariate analysis but no significant correlation to be identified in the Cox multivariate analysis. Undertaking further research by expanding the sample size and conducting a multi-center case comparison would be helpful to obtain a more accurate conclusion in this aspect.

The above results revealed that, as a cancer promoting factor, Cx43 participates in the epithelial-mesenchymal transition process in CTCs, promotes the distant metastasis of tumor cells, and causes disease progression. Its expression may be related to the expression of Her2. Cx43 expression in CTCs indicates a poor prognosis for patients.

At present, in the vast majority of studies, the detection of Cx43 is based on samples from primary or distant breast cancer metastases. These tissues are more difficult to obtain than blood samples. The method of detecting Cx43 in CTCs has the advantages of sample availability and timeliness when compared to sampling original tumor lesions. Patients merely need to have their blood drawn, which makes detection and promotion easier in clinical practice. Therefore, the presence of Cx43 in CTCs can be used as an indicator to assess the risk of breast cancer recurrence. If Cx43 is expressed, more aggressive clinical interventions should be employed.

In conclusion, we found that the expression of Cx43 in CTCs is associated with advanced disease, metastasis, and poor survival in patients with breast cancer. Univariate Cox regression analysis revealed a significant association between Cx43 expression in CTCs and DFS. Our findings also indicate that assessment of Cx43 expression in CTCs may be a novel and promising strategy for metastasis evaluation in patients with breast cancer. Furthermore, we found higher Cx43 expression in the Her2 overexpression group. Since our study cohort was limited in size, our current findings need to be confirmed with further large-scale studies. Our data only reveals a trend, and we did not further investigate the deeper relationship between Cx43 and Her2. Furthermore, it will be critical to explore potential correlations between specific expression levels of Cx43 and genotypes, as well as responses to therapy and survival in patients with breast cancer.

Ethics approval and consent to participate

This study was conducted with approval from the Ethics Committee of The Second Affiliated Hospital of Chongqing Medical University (2022–19). This study was conducted in accordance with the declaration of Helsinki and the study complies with all regulations. Written informed consent was obtained from all participants.

Author contribution statement

Dan-Qing Wang: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Jia Ming: Conceived and designed the experiments; Wrote the paper.
Yuan-Yuan Wang: Performed the experiments; Analyzed and interpreted the data.
Yan-Ling Shi; Bin Zeng; Zi-Jing Lin; Qin Deng: Performed the experiments.

Funding statement

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviation

Cx43   Connexins 43
CTC   circulating tumor cell
ER   estrogen receptor
PR   progesterone receptor
Ki-67   nuclear-associated antigen

Her2   human epidermal growth factor receptor
C-erbB-2 Tyrosine kinase-type cell surface receptor Her2
TMN   tumor node metastasis classification
DFS   disease free survival
ISH   in situ hybridization
FISH   fluorescence in situ hybridization
EpCAM   Epithelial cell adhesion molecule

References

[1] M.M. Cao, W.Q. Chen, Interpretation on the global cancer statistics of GLOBOCAN 2020, Chin J Front Med Sci Electron Version 13 (3) (2021) 63–69.

[2] F. Bray, J. Ferlay, I. Soerjomataram, et al., Global cancer statistics 2018: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries, Ca - Cancer J. Clin. 68 (6) (2018) 394–424, https://doi.org/10.3322/caac.21492.

[3] T. Li, C. Mello-Thoms, P.C. Brennan, Descriptive epidemiology of breast cancer in China: incidence, mortality, survival and prevalence, Breast Cancer Res. Treat. 159 (3) (2016) 395–406, https://doi.org/10.1007/s10549-016-3947-0.

[4] T. Zuo, H. Zeng, H. Li, et al., The influence of stage at diagnosis and molecular subtype on breast cancer patient survival: a hospitalbased multi-center study, Chin. J. Cancer 36 (1) (2017) 84.

[5] F. De Luca, G. Rotunno, B.S. Wittner, et al., Mutational analysis of single circulating tumor cells by next generation sequencing in metastatic breast cancer, Oncotarget 7 (18) (2016) 26107–26119.

[6] T. Masuda, N. Hayashi, T. Iguchi, et al., Clinical and biological significance of circulating tumor cells in cancer, Mol. Oncol. 10 (3) (2016) 408–417.

[7] M. Cristofanilli, D.F. Hayes, G.T. Budd, M.J. Ellis, A. Stopeck, J.M. Reuben, G.V. Doyle, J. Matera, W.J. Allard, M.C. Miller, H.A. Fritsche, G.N. Hortobagyi, L. W. Terstappen, Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer, J. Clin. Oncol. 23 (7) (2005 Mar 1) 1420–1430, https://doi.org/10.1200/JCO.2005.08.140.

[8] C.L. Grek, J.M. Rhett, G.S. Ghatnekar, Cardiac to cancer: connecting connexins to clinical opportunity, FEBS Lett. 588 (8) (2014 Apr 17) 1349–1364, https://doi.org/10.1016/j.febslet.2014.02.047.

[9] N.M. Kumar, N.B. Gilula, The gap junction communication channel, Cell 84 (3) (1996 Feb 9) 381–388, https://doi.org/10.1016/s0092-8674(00)81282-9.

[10] L. Makowski, D.L. Caspar, W.C. Phillips, D.A. Goodenough, Gap junction structures. II. Analysis of the x-ray diffraction data, J. Cell Biol. 74 (2) (1977 Aug) 629–645, https://doi.org/10.1083/jcb.74.2.629.

[11] J.M. Rhett, S.A. Fann, M.J. Yost, Purinergic signaling in early inflammatory events of the foreign body response: modulating extracellular ATP as an enabling technology for engineered implants and tissues, Tissue Eng. B Rev. 20 (5) (2014 Oct) 392–402, https://doi.org/10.1089/ten.TEB.2013.0554.

[12] Y. Ding, T.A. Nguyen, PQ1, a quinoline derivative, induces apoptosis in T47D breast cancer cells through activation of caspase-8 and caspase-9, Apoptosis 18 (9) (2013 Sep) 1071–1082, https://doi.org/10.1007/s10495-013-0855-1.

[13] Y. Ding, K. Prasain, T.D. Nguyen, D.H. Hua, T.A. Nguyen, The effect of the PQ1 anti-breast cancer agent on normal tissues, Anti Cancer Drugs 23 (9) (2012 Oct) 897–905, https://doi.org/10.1097/CAD.0b013e328354ac71.

[14] S.N. Shishido, A. Delahaye, A. Beck, T.A. Nguyen, The anticancer effect of PQ1 in the MMTV-PyVT mouse model, Int. J. Cancer 134 (6) (2014 Mar 15) 1474–1483, https://doi.org/10.1002/ijc.28461. Epub 2013 Sep. 19.

[15] C.L. Grek, J.M. Rhett, J.S. Bruce, M.A. Abt, G.S. Ghatnekar, E.S. Yeh, Targeting connexin 43 with α-connexin carboxyl-terminal (ACT1) peptide enhances the activity of the targeted inhibitors, tamoxifen and lapatinib, in breast cancer: clinical implication for ACT1, BMC Cancer 15 (2015 Apr 3) 296, https://doi.org/10.1186/s12885-015-1229-6.

[16] C.L. Grek, J.M. Rhett, J.S. Bruce, G.S. Ghatnekar, E.S. Yeh, Connexin 43, breast cancer tumor suppressor: missed connections? Cancer Lett. 374 (1) (2016 Apr 28) 117–126, https://doi.org/10.1016/j.canlet.2016.02.008.

[17] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, F. Bray, A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, 2015, Ca - Cancer J. Clin. 66 (2) (2016 Mar-Apr) 115–132, https://doi.org/10.3322/caac.21338.

[18] F. Puglisi, Locoregional recurrence of triple-negative breast cancer after breast-conserving surgery and radiation, Cancer 116 (2) (2010 Jan 15) 538, https://doi.org/10.1002/cncr.24826, author reply 538-9.

[19] R. Badwe, S. Gupta, N. Nair, V. Parmar, R. Hawaldar, Survival of patients with metastatic breast cancer with or without locoregional therapy - authors' reply, Lancet Oncol. 16 (16) (2015 Dec) e587–e588, https://doi.org/10.1016/S1470-2045(15)00479-9.

[20] M. Sundquist, L. Brudin, G. Tejler, Improved survival in metastatic breast cancer 1985-2016, Breast 31 (2017 Feb) 46–50, https://doi.org/10.1016/j.breast.2016.10.005.

[21] F.C. Bidard, D.J. Peeters, T. Fehm, F. Nolé, R. Gisbert-Criado, D. Mavroudis, S. Grisanti, D. Generali, J.A. Garcia-Saenz, J. Stebbing, C. Caldas, P. Gazzaniga, L. Manso, R. Zamarchi, A.F. de Lascoiti, L. De Mattos-Arruda, M. Ignatiadis, R. Lebofsky, S.J. van Laere, F. Meier-Stiegen, M.T. Sandri, J. Vidal-Martinez, E. Politaki, F. Consoli, A. Bottini, C. Diaz-Rubio, J. Krell, S.J. Dawson, C. Raimondi, A. Rutten, W. Janni, E. Munzone, V. Carañana, C. Agelaki, C. Almici, L. Dirix, E.F. Solomayer, L. Zorzino, H. Johannes, J.S. Reis-Filho, K. Pantel, J.Y. Pierga, S. Michiels, Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data, Lancet Oncol. 15 (4) (2014 Apr) 406–414, https://doi.org/10.1016/S1470-2045(14)70069-5.

[22] B. Rack, J. Jückstock, M. Günthner-Biller, U. Andergassen, J. Neugebauer, P. Hepp, A. Schoberth, D. Mayr, T. Zwingers, C. Schindlbeck, K. Friese, W. Janni, Trastuzumab clears HER2/neu-positive isolated tumor cells from bone marrow in primary breast cancer patients, Arch. Gynecol. Obstet. 285 (2) (2012 Feb) 485–492, https://doi.org/10.1007/s00404-011-1954-2.

[23] M. Wallwiener, A.D. Hartkopf, I. Baccelli, S. Riethdorf, S. Schott, K. Pantel, F. Marmé, C. Sohn, A. Trumpp, B. Rack, B. Aktas, E.F. Solomayer, V. Müller, W. Janni, A. Schneeweiss, T.N. Fehm, The prognostic impact of circulating tumor cells in subtypes of metastatic breast cancer, Breast Cancer Res. Treat. 137 (2) (2013 Jan) 503–510, https://doi.org/10.1007/s10549-012-2382-0.

[24] A. Giordano, M. Giuliano, M. De Laurentiis, G. Arpino, S. Jackson, B.C. Handy, N.T. Ueno, E. Andreopoulou, R.H. Alvarez, V. Valero, S. De Placido, G. N. Hortobagyi, J.M. Reuben, M. Cristofanilli, Circulating tumor cells in immunohistochemical subtypes of metastatic breast cancer: lack of prediction in HER2-positive disease treated with targeted therapy, Ann. Oncol. 23 (5) (2012 May) 1144–1150, https://doi.org/10.1093/annonc/mdr434.

[25] J.Y. Pierga, D. Hajage, T. Bachelot, S. Delaloge, E. Brain, M. Campone, V. Diéras, E. Rolland, L. Mignot, C. Mathiot, F.C. Bidard, High independent prognostic and predictive value of circulating tumor cells compared with serum tumor markers in a large prospective trial in first-line chemotherapy for metastatic breast cancer patients, Ann. Oncol. 23 (3) (2012 Mar) 618–624, https://doi.org/10.1093/annonc/mdr263.

[26] L. Cabel, C. Proudhon, H. Gortais, D. Loirat, F. Coussy, J.Y. Pierga, F.C. Bidard, Circulating tumor cells: clinical validity and utility, Int. J. Clin. Oncol. 22 (3) (2017 Jun) 421–430, https://doi.org/10.1007/s10147-017-1105-2.

[27] M. Labelle, S. Begum, R.O. Hynes, Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis, Cancer Cell 20 (5) (2011 Nov 15) 576–590, https://doi.org/10.1016/j.ccr.2011.09.009.

[28] Y. Kang, K. Pantel, Tumor cell dissemination: emerging biological insights from animal models and cancer patients, Cancer Cell 23 (5) (2013 May 13) 573–581, https://doi.org/10.1016/j.ccr.2013.04.017.

[29] C. Alix-Panabières, K. Pantel, Challenges in circulating tumour cell research, Nat. Rev. Cancer 14 (9) (2014 Sep) 623–631, https://doi.org/10.1038/nrc3820.

[30] M. Yu, A. Bardia, B.S. Wittner, S.L. Stott, M.E. Smas, D.T. Ting, S.J. Isakoff, J.C. Ciciliano, M.N. Wells, A.M. Shah, K.F. Concannon, M.C. Donaldson, L.V. Sequist, E. Brachtel, D. Sgroi, J. Baselga, S. Ramaswamy, M. Toner, D.A. Haber, S. Maheswaran, Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition, Science 339 (6119) (2013 Feb 1) 580–584, https://doi.org/10.1126/science.1228522.

[31] A. Lucci, C.S. Hall, A.K. Lodhi, A. Bhattacharyya, A.E. Anderson, L. Xiao, I. Bedrosian, H.M. Kuerer, S. Krishnamurthy, Circulating tumour cells in non-metastatic breast cancer: a prospective study, Lancet Oncol. 13 (7) (2012 Jul) 688–695, https://doi.org/10.1016/S1470-2045(12)70209-7.

[32] F.C. Bidard, S. Michiels, S. Riethdorf, V. Mueller, L.J. Esserman, A. Lucci, B. Naume, J. Horiguchi, R. Gisbert-Criado, S. Sleijfer, M. Toi, J.A. Garcia-Saenz, A. Hartkopf, D. Generali, F. Rothé, J. Smerage, L. Muinelo-Romay, J. Stebbing, P. Viens, M.J.M. Magbanua, C.S. Hall, O. Engebraaten, D. Takata, J. Vidal-Martínez, W. Onstenk, N. Fujisawa, E. Diaz-Rubio, F.A. Taran, M.R. Cappelletti, M. Ignatiadis, C. Proudhon, D.M. Wolf, J.B. Bauldry, E. Borgen, R. Nagaoka, V. Carañana, J. Kraan, M. Maestro, S.Y. Brucker, K. Weber, F. Reyal, D. Amara, M.G. Karhade, R.R. Mathiesen, H. Tokiniwa, A. Llombart-Cussac, A. Meddis, P. Blanche, K. d'Hollander, P. Cottu, J.W. Park, S. Loibl, A. Latouche, J.Y. Pierga, K. Pantel, Circulating tumor cells in breast cancer patients treated by neoadjuvant chemotherapy: a meta-analysis, J. Natl. Cancer Inst. 110 (6) (2018 Jun 1) 560–567, https://doi.org/10.1093/jnci/djy018.

[33] B. Rack, C. Schindlbeck, J. Jückstock, U. Andergassen, P. Hepp, T. Zwingers, T.W. Friedl, R. Lorenz, H. Tesch, P.A. Fasching, T. Fehm, A. Schneeweiss, W. Lichtenegger, M.W. Beckmann, K. Friese, K. Pantel, W. Janni, SUCCESS Study Group, Circulating tumor cells predict survival in early average-to-high risk breast cancer patients, J. Natl. Cancer Inst. 106 (5) (2014 May 15) dju066, https://doi.org/10.1093/jnci/dju066.

[34] L. Kańczuga-Koda, M. Sulkowska, M. Koda, J. Reszeć, W. Famulski, M. Baltaziak, S. Sulkowski, Expression of connexin 43 in breast cancer in comparison with mammary dysplasia and the normal mammary gland, Folia Morphol. 62 (4) (2003 Nov) 439–442. PMID: 14655136.

[35] L. Kanczuga-Koda, M. Sulkowska, M. Koda, R. Rutkowski, S. Sulkowski, Increased expression of gap junction protein–connexin 32 in lymph node metastases of human ductal breast cancer, Folia Histochem. Cytobiol. 45 (Suppl 1) (2007) S175–S180. PMID: 18292829.

[36] L. Kanczuga-Koda, S. Sulkowski, A. Lenczewski, M. Koda, A. Wincewicz, M. Baltaziak, M. Sulkowska, Increased expression of connexins 26 and 43 in lymph node metastases of breast cancer, J. Clin. Pathol. 59 (4) (2006 Apr) 429–433, https://doi.org/10.1136/jcp.2005.029272. PMID: 16567471.

[37] L. Kanczuga-Koda, S. Sulkowski, J. Tomaszewski, M. Koda, M. Sulkowska, W. Przystupa, J. Golaszewska, M. Baltaziak, Connexins 26 and 43 correlate with Bak, but not with Bcl-2 protein in breast cancer, Oncol. Rep. 14 (2) (2005 Aug) 325–329. PMID: 16012710.

[38] D.W. Laird, P. Fistouris, G. Batist, L. Alpert, H.T. Huynh, G.D. Carystinos, M.A. Alaoui-Jamali, Deficiency of connexin43 gap junctions is an independent marker for breast tumors, Cancer Res. 59 (16) (1999 Aug 15) 4104–4110. PMID: 10463615.

[39] M.K. Elzarrad, A. Haroon, K. Willecke, R. Dobrowolski, M.N. Gillespie, A.B. Al-Mehdi, Connexin-43 upregulation in micrometastases and tumor vasculature and its role in tumor cell attachment to pulmonary endothelium, BMC Med. 6 (2008 Jul 22) 20, https://doi.org/10.1186/1741-7015-6-20. PMID: 18647409; PMCID: PMC2492868.

[40] Y. Chao, Q. Wu, M. Acquafondata, R. Dhir, A. Wells, Partial mesenchymal to epithelial reverting transition in breast and prostate cancer metastases, Cancer Microenviron 5 (1) (2012 Apr) 19–28, https://doi.org/10.1007/s12307-011-0085-4. Epub 2011 Sep 3. PMID: 21892699; PMCID: PMC3343195.

[41] A. Ito, K. Watabe, Y. Koma, Y. Kitamura, An attempt to isolate genes responsible for spontaneous and experimental metastasis in the mouse model, Histol. Histopathol. 17 (3) (2002) 951–959, https://doi.org/10.14670/HH-17.951. PMID: 12168807.

[42] J.L. Solan, S.R. Hingorani, P.D. Lampe, Changes in connexin43 expression and localization during pancreatic cancer progression, J. Membr. Biol. 245 (5–6) (2012 Jun) 255–262, https://doi.org/10.1007/s00232-012-9446-2. Epub 2012 Jun 23. PMID: 22729649; PMCID: PMC3518378.

[43] Q. Shao, H. Wang, E. McLachlan, G.I. Veitch, D.W. Laird, Down-regulation of Cx43 by retroviral delivery of small interfering RNA promotes an aggressive breast cancer cell phenotype, Cancer Res. 65 (7) (2005 Apr 1) 2705–2711, https://doi.org/10.1158/0008-5472.CAN-04-2367. PMID: 15805269.

[44] Z. Li, Z. Zhou, H.J. Donahue, Alterations in Cx43 and OB-cadherin affect breast cancer cell metastatic potential, Clin. Exp. Metastasis 25 (3) (2008) 265–272, https://doi.org/10.1007/s10585-007-9140-4. Epub 2008 Jan 10. PMID: 18193170.

[45] E. McLachlan, Q. Shao, H.L. Wang, S. Langlois, D.W. Laird, Connexins act as tumor suppressors in three-dimensional mammary cell organoids by regulating differentiation and angiogenesis, Cancer Res. 66 (20) (2006 Oct 15) 9886–9894, https://doi.org/10.1158/0008-5472.CAN-05-4302. PMID: 17047050.

[46] I. Plante, M.K. Stewart, K. Barr, A.L. Allan, D.W. Laird, Cx43 suppresses mammary tumor metastasis to the lung in a Cx43 mutant mouse model of human disease, Oncogene 30 (14) (2011 Apr 7) 1681–1692, https://doi.org/10.1038/onc.2010.551. Epub 2010 Dec 13. PMID: 21151177.

[47] Z. Li, Z. Zhou, D.R. Welch, H.J. Donahue, Expressing connexin 43 in breast cancer cells reduces their metastasis to lungs, Clin. Exp. Metastasis 25 (8) (2008) 893–901, https://doi.org/10.1007/s10585-008-9208-9. Epub 2008 Oct 7. PMID: 18839320; PMCID: PMC2754227.

[48] W.K. Wang, M.C. Chen, H.F. Leong, Y.L. Kuo, C.Y. Kuo, C.H. Lee, Connexin 43 suppresses tumor angiogenesis by down-regulation of vascular endothelial growth factor via hypoxic-induced factor-1α, Int. J. Mol. Sci. 16 (1) (2014 Dec 26) 439–451, https://doi.org/10.3390/ijms16010439. PMID: 25548899; PMCID: PMC4307255.

[49] S. Valastyan, R.A. Weinberg, Tumor metastasis: molecular insights and evolving paradigms, Cell 147 (2) (2011 Oct 14) 275–292, https://doi.org/10.1016/j.cell.2011.09.024. PMID: 22000009; PMCID: PMC3261217.

[50] A.D. Rhim, E.T. Mirek, N.M. Aiello, A. Maitra, J.M. Bailey, F. McAllister, M. Reichert, G.L. Beatty, A.K. Rustgi, R.H. Vonderheide, S.D. Leach, B.Z. Stanger, EMT and dissemination precede pancreatic tumor formation, Cell 148 (1–2) (2012 Jan 20) 349–361, https://doi.org/10.1016/j.cell.2011.11.025. PMID: 22265420; PMCID: PMC3266542.

[51] A. Genna, A.M. Vanwynsberghe, A.V. Villard, C. Pottier, J. Ancel, M. Polette, C. Gilles, EMT-associated heterogeneity in circulating tumor cells: sticky friends on the road to metastasis, Cancers 12 (6) (2020 Jun 19) 1632, https://doi.org/10.3390/cancers12061632. PMID: 32575608; PMCID: PMC7352430.