Circulating tumor cell heterogeneity and MMP9 expression in clinical correlation with breast cancer

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Abstract

Purpose: This study aimed to investigate the association between the heterogeneity of circulating tumor cell (CTC) subtypes and the expression patterns of matrix metalloproteinase 9 (MMP9) with clinicopathological parameters in breast cancer patients. The distribution of CTC subtypes, MMP9 expression levels, and their influence on tumor progression were further explored.

Methods: A total of 303 breast cancer patients were enrolled in this study, and the analysis of circulating tumor cells (CTCs) and their subtypes in peripheral blood was integrated with the assessment of matrix metalloproteinase 9 (MMP9) expression.

Results: The number of mesenchymal CTCs increased significantly with the number of lymph node metastases ($P < 0.05$), and high MMP9 expression was positively correlated with lymph node involvement ($P < 0.05$). The proportion of intermediate to high MMP9 expression was significantly higher in mixed CTCs compared to epithelial and mesenchymal CTCs ($P < 0.05$). High MMP9 expression in tumor tissues was associated with increased CTCs production and induction of epithelial-mesenchymal transition (EMT) ($P < 0.05$), as well as an elevated number of MMP9-positive CTCs ($P < 0.05$).

Conclusions: These findings suggest that MMP9 may promote CTC shedding and the EMT process by degrading the extracellular matrix.

1. Introduction

As a malignant tumor with the highest incidence rate among women worldwide,

breast cancer incidence rate continues to climb, showing a trend among younger age groups (1–4). The risk of breast cancer is markedly elevated in women with prolonged menstrual history, nulliparity, and advanced age at first childbirth. As a systemic disease, breast cancer has the potential to metastasize via both lymphatic and hematogenous routes even at early stages. The most common sites of metastasis include the bones, lungs, liver, and brain(5–7). Circulating tumor cells (CTCs), shed from primary tumors or metastatic sites, enter the peripheral circulation and disseminate to colonize distant organs. CTCs thus represent dynamic biomarkers of tumor invasion and metastatic progression(8,9). Epithelial-mesenchymal transition (EMT) confers mesenchymal traits upon CTCs, enabling basement membrane breaching, enhanced migratory capacity, and immune evasion(10–12).

Matrix metalloproteinases (MMPs) constitute a family of zinc-dependent endopeptidases that degrade extracellular matrix (ECM) constituents. These enzymes participate in physiological processes while contributing to pathological conditions such as arthritis and oncogenesis. MMPs facilitate tumor invasion and metastasis through three principal mechanisms: ECM degradation, activation of oncogenic signaling pathways, and induction of EMT. Among this family, MMP9 plays a critical role in metastatic progression and serves as a key clinical biomarker for assessing breast cancer prognosis(13–16). However, the mechanistic relationship linking MMP9 and CTCs in clinical samples is not fully elucidated.

This study characterized subtypes of peripheral blood CTCs, total CTC counts, and MMP9 expression in breast cancer patients. Our study systematically assessed the correlations between CTC heterogeneity and clinicopathological characteristics, associations of MMP9 expression with clinicopathological parameters, and interrelationships among CTC subtypes, total CTC counts, and MMP9 expression. These findings establish a theoretical framework for developing metastasis risk stratification models and optimizing personalized targeted therapies.

2. Methods

Patients. A total of 303 breast cancer patients were included in this study (Table 1). Blood or tissue samples were collected for CTC analyses or immunohistochemical staining (Table 1). All patients were diagnosed and treated between August 1, 2017, and July 6, 2018, at Nanfang Hospital of Southern Medical University. Pathological data, such as patients age, sex, tumor diameter, lymph node metastasis, Ki-67, and HER-2 status were recorded in the study. Clinical staging was determined according to the 8th edition of the AJCC breast cancer staging system. The study protocol was approved by the Ethics Committee of Nanfang Hospital.

Table 1. Patients Characteristic

Study Group	Number of Patients (Cases)	Inclusion Criteria	Exclusion Criteria

CTC Heterogeneity Group	111 cases	1. Diagnosed with breast malignancy via core needle biopsy under ultrasound guidance, pathological examination, or consultation of external hospital pathological slides; 2. Complete medical records; Preoperative routine examinations performed at our hospital showed no distant metastasis and the patient was eligible for surgery; 3. First diagnosis of breast malignancy, without prior local surgical treatment such as modified radical mastectomy or breast-conserving surgery, and without prior comprehensive therapies like chemotherapy, radiotherapy, or targeted therapy; 4. Aged ≥18 years and provided informed consent.	1. Patients with a history of malignancy in other sites; 2. Patients suspected of having malignancy in other sites upon this admission; 3. Patients with incomplete clinical or imaging data; 4. Patients who had undergone tumor resection surgery or received neoadjuvant chemotherapy, radiotherapy, or other related treatments.

MMP9 Expression Group	116 cases	1.Diagnosed with breast malignancy via core needle biopsy under ultrasound guidance, pathological examination, or consultation of external hospital pathological slides; 2. Complete medical records; Preoperative routine examinations performed at our hospital provided baseline data; 3. Aged ≥18 years and provided informed consent.	1. Patients with a history of malignancy in other sites; 2. Patients suspected of having malignancy in other sites upon this admission; 3. Patients with incomplete clinical or imaging data.
CTC-MMP9 Correlation Group	76 cases	1. Diagnosed with breast malignancy via core needle biopsy under ultrasound guidance, pathological examination, or consultation of external hospital pathological slides; 2. Complete medical records; Preoperative routine examinations performed at our hospital showed no distant metastasis and the patient was eligible for	1. Patients with a history of malignancy in other sites; 2. Patients suspected of having malignancy in other sites upon this admission; 3. Patients with incomplete clinical or

	surgery; 3. First diagnosis of breast malignancy, without prior local surgical treatment such as breast-conserving surgery or modified radical mastectomy, and without prior comprehensive therapies; 4. Aged ≥18 years and provided informed consent.	imaging data; 4. Patients who had undergone tumor resection surgery or received neoadjuvant chemotherapy, radiotherapy, or other related treatments.

CTCs detection. Peripheral venous blood was collected early in the morning before surgery, and CTCs were detected by CanPatrol™ second-generation CTC detection technology (17), CK18, CK19, EpCAM, and CK8 labeled epithelial CTCs, twist, and vimentin labeled mesenchymal CTCs, and CD45 labeled leukocytes; Epithelial CTCs were labeled with fluorescent dyes with red signals, mesenchymal CTCs were labeled with green fluorescent dyes, and mixed CTCs were labeled with both red and green dyes, and CTCs subtypes were counted and analyzed separately. Individual CTCs were labeled with MMP9 probe, and MMP9 probe was labeled with purple fluorescent dye.

Immunohistochemical (IHC) staining and fluorescence in situ hybridization (FISH). The slides were analyzed in a double-blind manner by two independent pathologists. Scores were assigned based on the intensity of positive staining: 1 for weak positivity (+), 2 for moderate positivity (++), and 3 for strong positivity (+++). The formula for scoring was calculated as follows: (+) %×1 + (++) %×2 + (+++) %×3. A total score of less than 1.0 was classified as (+), 1.0 to 1.5 as (++), and greater than 1.5 as (+++).

To determine HER2 status in breast cancer, 2013 ASCO/CAP guidelines was followed(18). HER-2/CEP17 ratio ≥ 2.0 or mean HER-2 copy number/cell ≥ 6.0 was determined as HER-2 positive; HER-2/CEP17 ratio < 2.0 and mean HER-2 copy number/cell < 4.0 was determined as HER-2 negative. HER-2/CEP17 ratio < 2.0, mean HER-2 copy number/cell < 6.0, and ≥ 4.0 could not be determined as HER-2 results, combined with IHC results or re-selected other tissue blocks for examination; HER-2/CEP17 ratio ≥ 2.0 and mean HER-2 copy number/cell < 4.0, was controversial and required further interpretation based on the immunohistochemistry results, along with communication with the patient.

Ten consecutive fields of view were observed under a 400x microscope to derive

the respective proportions of MMP9 no expression, low expression, medium expression, and high expression, which were multiplied by their weights. The sums were (+) for less than 1.0, (++) for between 1.0 and 1.5, and (+++) for greater than 1.5.

Statistical software and methods. Statistical analyses were performed using SPSS 20.0 software. Comparisons between groups were conducted using the independent samples *t-test*, ANOVA, or nonparametric tests. Categorical variables were presented as frequencies (percentages), and the chi-square test or Fisher's exact test was applied. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Correlation of CTCs heterogeneity with clinical features

A total of 111 patients with invasive breast cancer were enrolled in this phase of the study (Table 1), with a mean age of 49.82 ± 9.63 years, ranging from 28 to 80 years. The clinical stages were as follows: 34 patients (30.6%) were in stage IA, 20 patients (18%) were in stage IB, 32 patients (28.8%) were in stage IIA, 11 patients (9.9%) were in stage IIB, 5 patients (4.5%) were in stage IIIA, 7 patients (6.3%) were in stage IIIB, and 2 patients (1.8%) were in stage IIIC. Among these patients, 110 were female and one was male.

CTCs were identified in 99 patients (89.19%), with a total of 593 CTCs detected, including 144 epithelial CTCs (24.28%), 319 mixed CTCs (53.79%), and 130 mesenchymal CTCs (21.92%). The mean total number of CTCs was 5.31±5.89, with 1.2±1.66 epithelial CTCs, 2.86±3.79 mixed CTCs, and 1.16±2.20 mesenchymal CTCs (Fig.1A).

To explore potential clinical correlates, we assessed the relationship between CTC counts and clinical stage.There were significant differences in the distribution of the total number of CTCs and mixed CTCs at different clinical stages ($P < 0.05$). Although the mean values of epithelial and mesenchymal CTCs were higher in stages II and III than those in stage I, the differences between the different clinical stages were not statistically significant ($P > 0.05$; Table 2).

Table 2. Comparison of CTCs distribution in different clinical stages

Clinical Stage	Stage I (n=54)	Stage II/III (n=57)	*t*	*P*
Total CTCs	3.93±3.34	6.61±7.35	7.7	0.014*
Epithelial CTCs	1.07±1.20	1.47±1.99	6.02	0.201
Mixed CTCs	2.04±2.16	3.65±4.74	10.62	0.023*
Mesenchymal CTCs	0.81±1.08	1.49±2.85	4.65	0.1

CTCs units: cells/5mL, expressed as mean ± standard deviation; * indicates P < 0.05, indicating a statistically significant difference.

Total number of CTCs in the group with a tumor diameter > 2 cm was statistically higher than that in the group with a tumor diameter ≤ 2 cm ($P < 0.05$). CTCs subtypes were not significantly differentially distributed between the two groups (all $P > 0.05$; Fig.1B).

Subsequently, the potential link with lymph node metastasis burden was examined. A total of 111 patients were categorized into the N0 group (64, 57.7%), N1 group (20, 18.0%), N2 group (16, 14.4%), and the N3 group (11, 9.9%) according to lymph node metastasis (Table 1). The Kruskal- Wallis test revealed significant differences in the distribution of total CTCs and mesenchymal CTCs among different lymph node metastasis groups (all $P < 0.05$), with both increasing as lymph node metastasis progressed. However, no statistically significant differences were observed in epithelial or mixed CTCs (both $P > 0.05$; Table 3).

Table 3. Comparison of CTCs distribution among different lymph node metastasis groups

Regional Lymph Nodes	N0 (n=64)	N1 (n=20)	N2 (n=16)	N3 (n=11)	*Z*	*P*
Total CTCs	3(0,14)	4(0,14)	4(0,25)	6(3,43)	7.983	0.046*
Epithelial CTCs	1(0,5)	0.5(0,5)	0.5(0,3)	1(0,11)	4.278	0.233
Mixed CTCs	1(0,9)	2(0,13)	2(0,22)	3(0,21)	4.887	0.18
Mesenchymal CTCs	0(0,9)	0(0,3)	0(0,4)	2(0,19)	9.371	0.025*

CTCs units: cells/5mL, expressed as median (minimum, maximum); * indicates $P < 0.05$, indicating a statistically significant difference between groups. N1: Metastasis in 1-3 ipsilateral axillary lymph nodes; N2: Metastasis in 4-9 ipsilateral axillary lymph nodes; N3: Metastasis in ≥10 ipsilateral axillary lymph nodes.

Among the 111 patients (Table 1), 38 patients (34.2%) were in the low-expression group, and 73 patients (65.8%) were in the high-expression group based on Ki-67 expression levels (20%). Significant differences were observed in the distribution of the total number of CTCs, as well as in the distribution of epithelial and mixed CTCs between the two groups ($P < 0.05$; Fig.1D).

In contrast to Ki-67 results, HER-2 status did not appear to strongly correlate with CTC profiles. There were 67 patients (60.91%) who were HER-2 negative and 43 HER-2 positive patients (39.09%) (Table 1). There were no significantly significant differences in the total number of CTCs or the distribution of any CTC subtypes between the HER-2 positive and negative groups (all $P > 0.05$; Fig.1E).

Finally, we investigated whether CTC patterns differed across intrinsic molecular subtypes of breast cancer. Patients were grouped according to the expression of HER-2, ER, PR, and Ki-67 status, with 30 cases of luminal A type (27.03%), 28 cases of luminal B HER-2 (-) type (25.22%), 26 cases of luminal B HER-2 (+) type (23.42%), HER-2 positive type was found in 17 cases (15.32%) and triple-negative type was found in 9 cases (8.11%). Because one patient showed (++) on HER-2 examination, FISH examination was not performed. This patient was not included in the subsequent statistical analyses. Kruskal-Wallis test showed that the total number of CTCs and the distribution of each subtype did not differ statistically among the different molecular typing groups (all $P > 0.05$; Table 4).

Table 4. Comparison of CTCs distribution among different molecular type groups

Molecular Type	Total	Epithelial	Mixed	Mesenchymal

	CTCs	CTCs	CTCs	CTCs
Luminal A	3 (0,12)	0 (0,5)	1 (0,9)	0 (0,9)
Luminal B HER-2 (-) Type	4.5 (0,43)	1 (0,11)	2 (0,13)	1 (0,19)
Luminal B HER-2 (+) Type	3 (0,25)	1 (0,5)	1 (0,22)	0.5 (0,4)
HER-2 Positive Type	6 (1,25)	1 (0,5)	3 (0,21)	2 (0,5)
Triple Negative Type	4 (0,7)	1 (0,4)	2 (0,4)	0 (0,1)
Z	8.447	6.783	6.961	6.878
P	0.077	0.148	0.138	0.142

One patient whose HER-2 test showed (++), but did not undergo supplementary FISH testing, was therefore excluded from the statistical grouping during analysis.

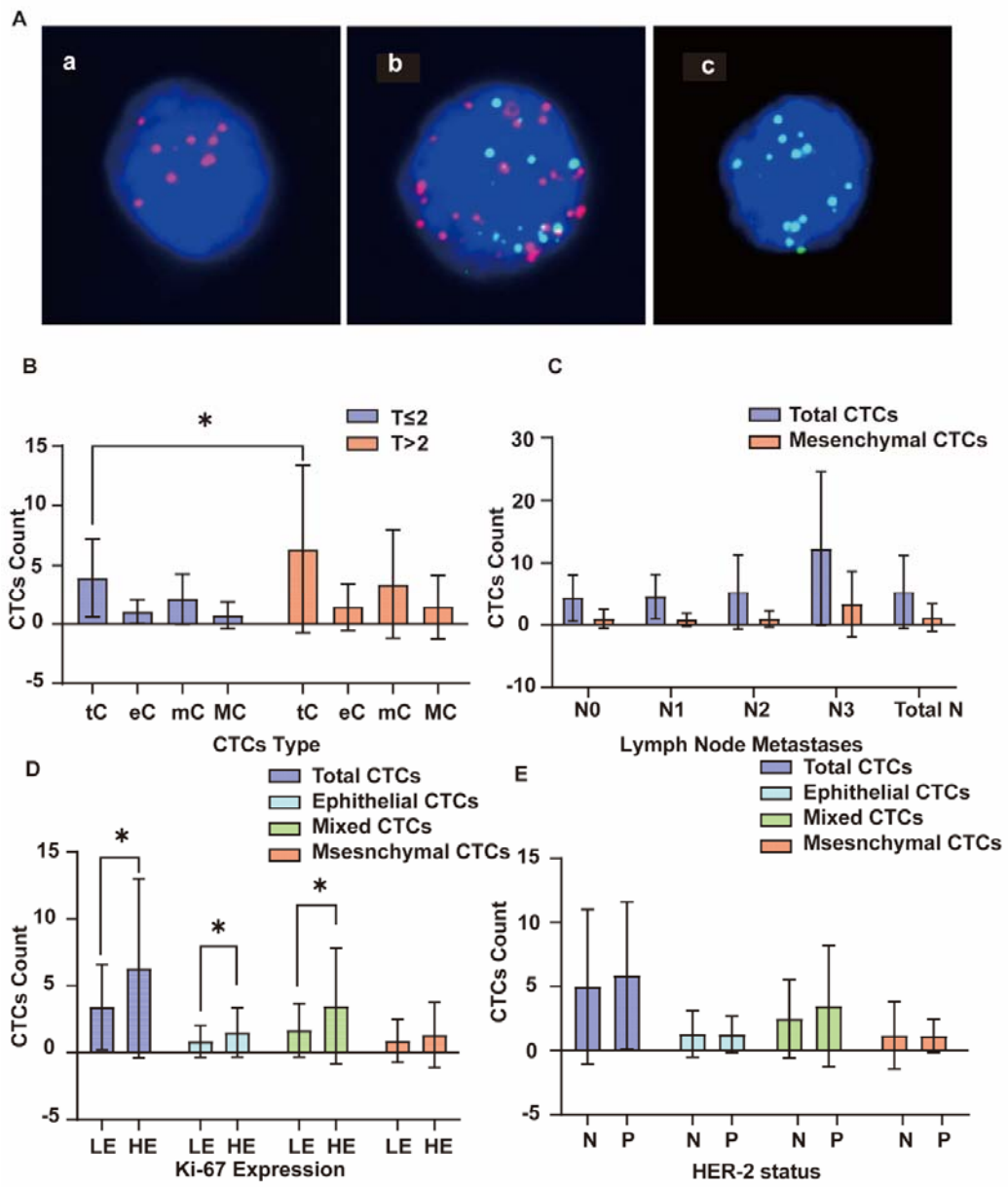

Fig 1 Correlation of CTCs heterogeneity with clinical features. (A) a: epithelial CTC shows red fluorescence signal; b: mixed CTC displays mixed red and green fluorescence signals; c: mesenchymal CTC displays green fluorescence signal. (B) The distribution of total CTCs (tc), epithelial CTCs (eC), mixed CTCs (mC), and mesenchymal CTCs (MC) in patients with tumor diameter ≤ 2 cm and > 2 cm. Intergroup comparisons were performed using independent samples t-tests, with homogeneity of variance assessed by Levene's test. The total CTC count was significantly lower in the ≤ 2cm group compared to the > 2cm group (t = 6.174, *P < 0.05). CTCs were quantified as counts/5ml. Error bars representing SD values. (C) The Kruskal-Wallis test revealed significant differences in the distribution of total CTCs and mesenchymal CTCs among different lymph node metastasis groups. N0: No regional lymph node metastasis. N1: Metastasis in ipsilateral peribronchial and/or

ipsilateral hilar lymph nodes, and intrapulmonary lymph node metastasis. N2: Metastasis in ipsilateral mediastinal and/or subcarinal lymph nodes. N3: Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, and supraclavicular lymph nodes. Error bars representing SD values. (D) Independent samples t-tests were performed to compare the differences in total CTCs and CTC subtype distributions between the high Ki-67 expression (HE) and low Ki-67 expression (LE) groups. Total CTCs, epithelial CTCs, and mixed CTCs were significantly higher in the HE group compared to the LE group (all $*P < 0.05$). Error bars representing SD values. (E) Independent samples t-tests were revealed no significant differences in either total CTCs or any CTC subtypes between HER-2 negative groups (N) and HER-2 positive groups (P) (all $P > 0.05$). Among the patients, one case with HER-2 (++) did not undergo FISH testing; two patients with intraductal carcinoma were not suitable for HER-2 testing. Error bars representing SD values.

3.2 Correlation between MMP9 expression and clinical features

A total of 116 breast cancer patients were enrolled, with an age range from 30 to 80 years and a median age of 50.56 ± 9.66 years (Table 1). Among these, 50 patients (43.10%) were lymph node-positive and 66 patients (56.90%) were lymph node-negative. The clinical stages were as follows: 2 patients (1.72%) were in Stage 0, 57 patients (49.14%) were in Stage I, 41 patients (35.34%) were in Stage II, 15 patients (12.93%) were in Stage III, and 1 patient (0.86%) was in Stage IV (Table 1).

Of the 116 patients (Table 1), 15 (12.9%) were weakly positive (+); 53 (45.7%) were moderately positive (++); and 48 (41.4%) were strongly positive (+++) (Fig.2A). Based on these expression levels, patients were categorized into two groups for comparison: the high-expression group (+++), which included 48 cases (41.38%) with a mean tumor diameter of 2.30±1.01 cm, and the low- and medium-expression group (+) and (++), which included 68 cases (58.62%) with a mean tumor diameter of 2.76±1.28 cm. Importantly, mean tumor diameter in the high-expression group was significantly smaller than in the low- and medium-expression groups ($P < 0.05$; Fig.2B).

The number of lymph node metastases in the three groups was 0.8±1.859 in the MMP9 (+) group, 3.64±5.463 in the MMP9 (++) group, and 2.06 ± 5.533 in the MMP9 (+++) group Critically, the difference in the number of lymph node metastases among the three groups was statistically significant ($P < 0.05$; Fig.2C).

Across the MMP9 expression groups (+), (++), (+++), the number of HER-2-negative patients consistently outnumbered that of HER-2-positive patients. Although the absolute number of negative patients increased with higher MMP9 expression levels, the proportion of HER-2-negative patients did not show a corresponding upward trend. Nevertheless, Statistical significance was observed among the groups ($P < 0.05$; Fig.2D).

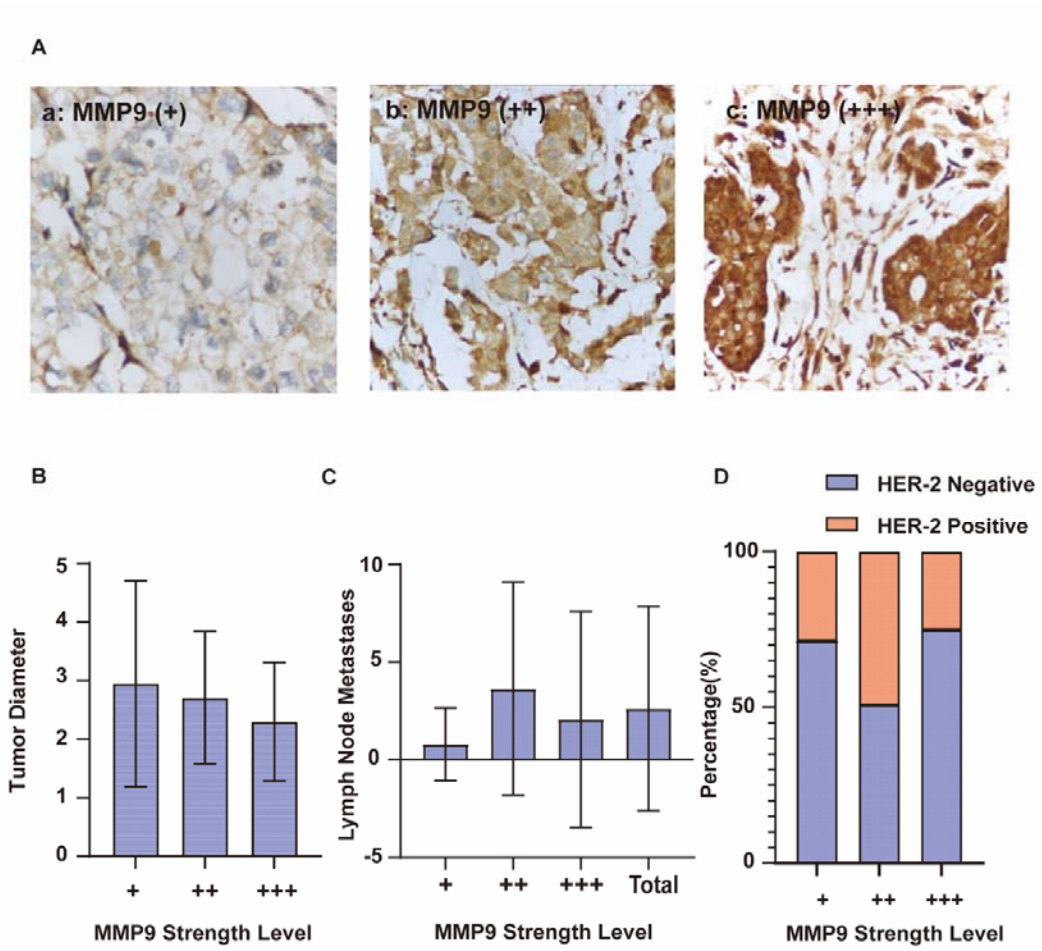

Fig 2 Correlation between MMP9 expression and clinical features. (A) MMP9 expression level example. (B) Independent samples t-tests were performed to the (+++) group had significantly smaller mean tumor diameter than the (+/++) group (t = 0.804, P = 0.039). Error bars representing SD values. (C) One-way ANOVA with Welch's correction (due to unequal variance, F = 3.266, P = 0.042) revealed significant differences in lymph node metastasis counts among MMP9 expression groups (F = 5.156, P = 0.008). Error bars representing SD values. (D) *Pearson's* χ^2 test revealed a significant association between MMP9 expression and HER-2 status (χ^2 = 6.582, P = 0.037). Among the patients, one case with HER-2 (++) did not undergo FISH testing, and two cases with ductal carcinoma in situ (DCIS) were unsuitable for HER-2 assessment.

3.3 Relationship between CTCs and MMP9 expression

A total of 76 patients with malignant breast tumors were included in the study (Table 1), with a mean age of 49.83±8.76 years, ranging from 30 to 69 years. Clinical stages were distributed as follows: Stage 0: 2 (2.63%), IA: 24 (31.58%), IB: 12 (15.79%), IIA: 26 (34.21%), IIB: 5 (6.58%), IIIA: 1 (1.32%), and IIIB: 6 (7.89%). Immunohistochemical examination of MMP9 in pathological sections revealed 19

cases (25%) of weak positivity (+), 45 cases (59%) of moderate positivity (++), and 12 cases (16%) of strong positivity (+++) in total (Table 1).

In both total CTCs and all CTC subtypes, CTCs counts of MMP9 expression (from negative to high) showed a progressive decrease (Fig.3A). We next analyzed CTC counts based on tumor tissue MMP9 status. Patients were divided into MMP9 (+) group (low-expression group) (19, 25.0%) and MMP9 (++) (+++) group (high-expression group) (57, 75.0%). The total number of mixed CTCs and CTCs was significantly higher in the high-expression group (both $P < 0.05$; Fig.3B), and there was no significant difference between the epithelial and mesenchymal CTCs groups (both $P > 0.05$; Fig.3B).

After grouping patients according to the strength of MMP9 expression in tumor tissues, analysis revealed that the frequency of MMP9 expression in CTCs decreased sequentially from no expression to high expression. The proportion of MMP9 expression in tumor tissues that constituted different levels of MMP9 expression in CTCs, MMP9 (++) and (+++) accounted for a relatively high percentage of the total number of MMP9 (++) and (+++).

However, the chi-square test showed that the difference between the groups was not statistically significant (*Pearson* χ^2=6.306, P=0.39; Fig.3C). Based on the grouping of MMP9 expression levels on CTCs, tumor tissue MMP9 was divided into a low expression group (MMP9 (+) group) and high expression group (MMP9 (++) (+++) group). Independent samples t-tests showed that the number of CTCs in the tumor tissue MMP9 high expression group was statistically higher than in the low-expression group when CTCs were lowly expressed in MMP9 ($P < 0.05$; Fig.3D).

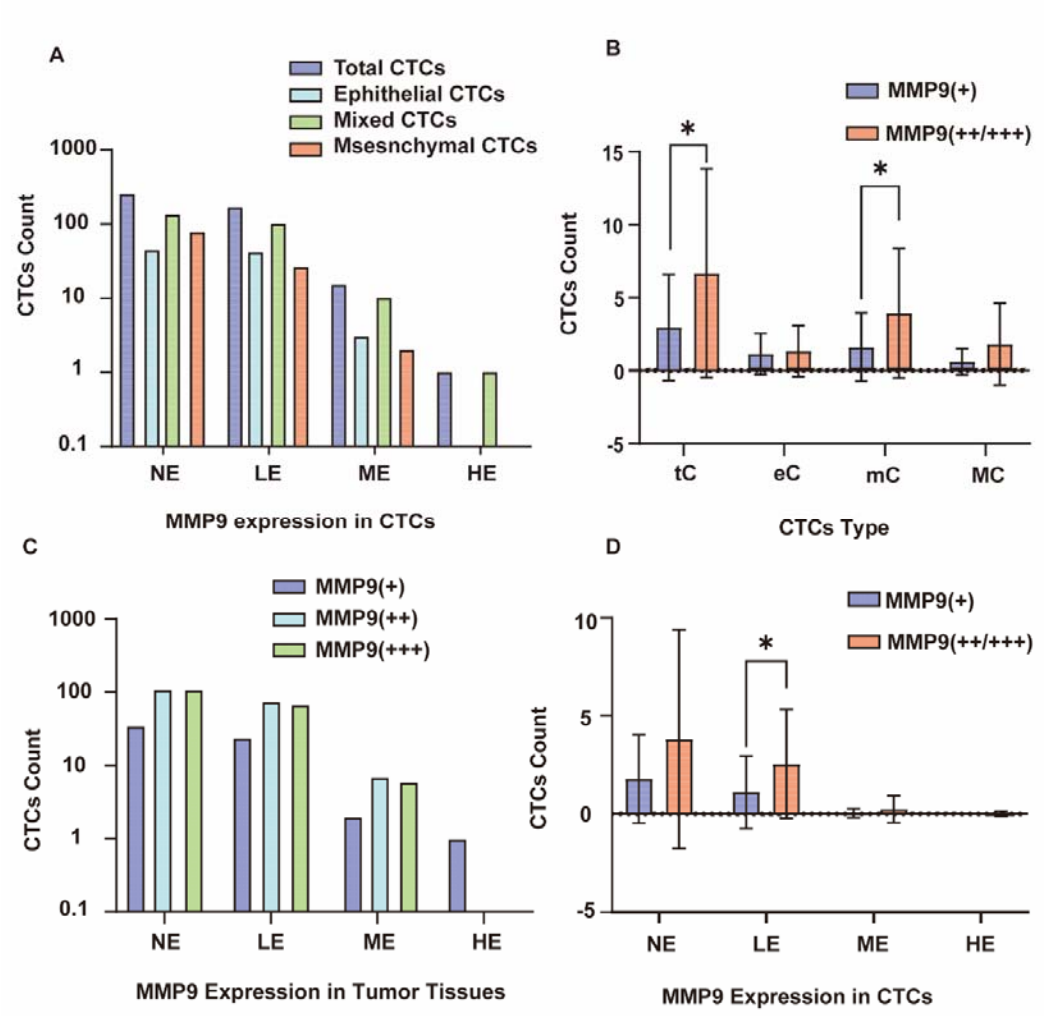

Fig 3 Relationship between CTCs and MMP9 expression. (A) Counts of each CTC subtype under different MMP9 expression levels. NE: no expression, LE: low expression, ME: medium expression, HE: high expression. CTCs counts were log10-transformed and plotted on a scale from 0.1 to 1000. (B) The distribution of total CTCs (tc), epithelial CTCs (eC), mixed CTCs (mC), and mesenchymal CTCs (MC) correlate with tumor MMP9 expression levels. Mixed CTCs and total CTCs were significantly higher in MMP9 (++/+++) than (+) groups (mixed CTCs: $t = 4.017$, $*P < 0.05$; total CTCs: $*P < 0.05$; *Welch's t-test* for mixed, standard *t-test* for total). Error bars representing SD values. (C) Analysis of CTC MMP9 expression by tumor MMP9 expression. NE: no expression, LE: low expression, ME: medium expression, HE: high expression. CTCs counts were log10-transformed and plotted on a scale from 0.1 to 1000. (D) Independent samples t-tests revealed significantly higher CTC counts in tumors with high MMP9 expression versus low MMP9 expression when CTCs exhibited low MMP9 expression ($t = 3.091$, $*P < 0.05$). Error bars representing SD values.

Discussion

The EMT phenotype of CTCs has been clinically validated as an important

prognostic indicator for breast cancer outcomes (19–21). Kalavska (22) employed RT-qPCR to quantify EMT-related transcripts in CTCs, revealing elevated MMP9 expression in aggressive breast cancer subtypes with potential clinical relevance. This study employed CanPatrol™ technology to achieve multidimensional phenotypic profiling of CTCs, overcoming limitations inherent to traditional CTC subtyping methodologies. This approach provides enhanced resolution of tumor heterogeneity and metastatic propensity. Furthermore, the simultaneous analysis of MMP9 expression in CTCs and tumor tissues revealed a correlation between CTC abundance and MMP9 expression.

Mesenchymal CTCs were prevalent in breast cancer patient groups and demonstrated significant correlation with metastatic progression. Pereira-Veiga(19) demonstrated that mesenchymal CTCs exhibit heightened invasive capacity but reduced proliferative potential. Ki-67, a key indicator of proliferative activity in breast cancer(23–25), has a central role in molecular typing, prognostic stratification, and treatment selection. In contrast, our analysis revealed significantly elevated CTC burden and increased proportions of mixed CTCs in patients with high Ki-67 expression, suggesting proliferative activity may potentiate CTC dissemination through enhanced tumor cell detachment or EMT induction. Detection of mixed and mesenchymal CTC phenotypes not only confirms active EMT in breast cancer, but also enables multimodal assessment of tumor progression, metastatic propensity, and proliferative activity through quantitative and phenotypic CTC dynamics. Elevated CTCs burden in peripheral blood has been documented in HER2-positive breast cancer patients(26); however, this study found no significant association between HER-2 status or molecular subtypes and CTC distribution. This null association may be attributable to limited sample size or intrinsic heterogeneity in breast cancer phenotypes. Although this component of the investigation employed a cross-sectional design that captures only a momentary assessment of EMT status, it establishes a foundational framework for future longitudinal studies evaluating dynamic correlations between CTC profiles and clinical prognosis.

We also found that MMP9 expression intensity was positively correlated with the number of lymph node metastases. Furthermore, study has demonstrated that high MMP9 expression is associated with lymph node metastasis and poorer prognosis in breast cancer(27). Paradoxically, tumors exhibiting strong MMP9 expression demonstrated significantly smaller diameters compared to moderately or weakly positive counterparts. This inverse correlation potentially reflects enhanced local invasive activity at tumor margins, constraining overall tumor expansion. Alternatively, limitations in immunohistochemical scoring system could contribute to this observation. The observed phenomenon suggests a dichotomous role for MMP9 in oncogenesis: in highly expressing tumors, MMP9 may facilitate hematogenous dissemination through activation of invasion-related signaling cascades, while tumors with attenuated MMP9 expression appear to favor primary tumor expansion, potentially through augmented proliferative signaling. This hypothesis is substantiated by clinical findings demonstrating attenuated proliferative activity in tumor cells with elevated MMP9 expression(22). Consequently, we postulate that MMP9 may function

as a "metastatic switch", whose expression threshold governs the transition from localized tumor proliferation to systemic dissemination.

This investigation demonstrated that elevated MMP9 expression in both tumor tissues and CTCs facilitates CTC detachment from primary sites and potentiates EMT activation. Similarly, elevated MMP9 expression in hepatocellular carcinoma (HCC) also promotes EMT activation and facilitates tumor cell invasion through basement membrane degradation(28). MMP9 deficiency dysregulates actin polymerization-related proteins, impairs cell migratory capacity, and attenuates TGF-β-induced EMT in lens epithelial cells(29). It is well-established that epithelial-mesenchymal transition (EMT) critically contributes to metastatic invasion in breast cancer(30–33). Shen et al. further demonstrated that 27-hydroxycholesterol promotes EMT via MMP9 upregulation(34), establishing a mechanistic pathway that leads to breast cancer invasion. Therefore, MMP9 represents a critical molecular determinant modulating phenotypic reprogramming in both EMT and CTCs, with its expression levels demonstrating significant correlations with CTC subtyping, quantitative abundance, and metastatic efficiency.

In summary, CTC heterogeneity and MMP9 overexpression demonstrate significant associations with clinicopathological characteristics in breast cancer. MMP9 may potentiate CTC metastatic competence through EMT induction while representing a critical regulator of tumor dissemination. Future prospective studies with larger patient groups are warranted to validate its clinical utility.

4. Conclusions

This study comprehensively investigates the heterogeneity of breast cancer CTCs, the EMT process, and the clinical relevance of MMP9 expression from multiple perspectives, providing a new theoretical basis for precision diagnosis and treatment of breast cancer.

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Availability of data and materials

All datasets generated and analyzed during the present study are included in this published article.

Authors' contributions

Y.M. designed the study and performed the experiments. S.C.B. performed the experiments. L.M.X. participated in CTCs detection. L.M.X., L.J.H., W.J.X., L.J.P., Z.H.Q., L.S.T., T.M.Y., participated in HIC. Y.M. and S.C.B. performed data analyses and interpretation. Y.M. and S.C.B. wrote the manuscript. Y.Y. , L.X. and M.Z.C. read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from the individual(s) for the publication of

any potentially identifiable images or data included in this article.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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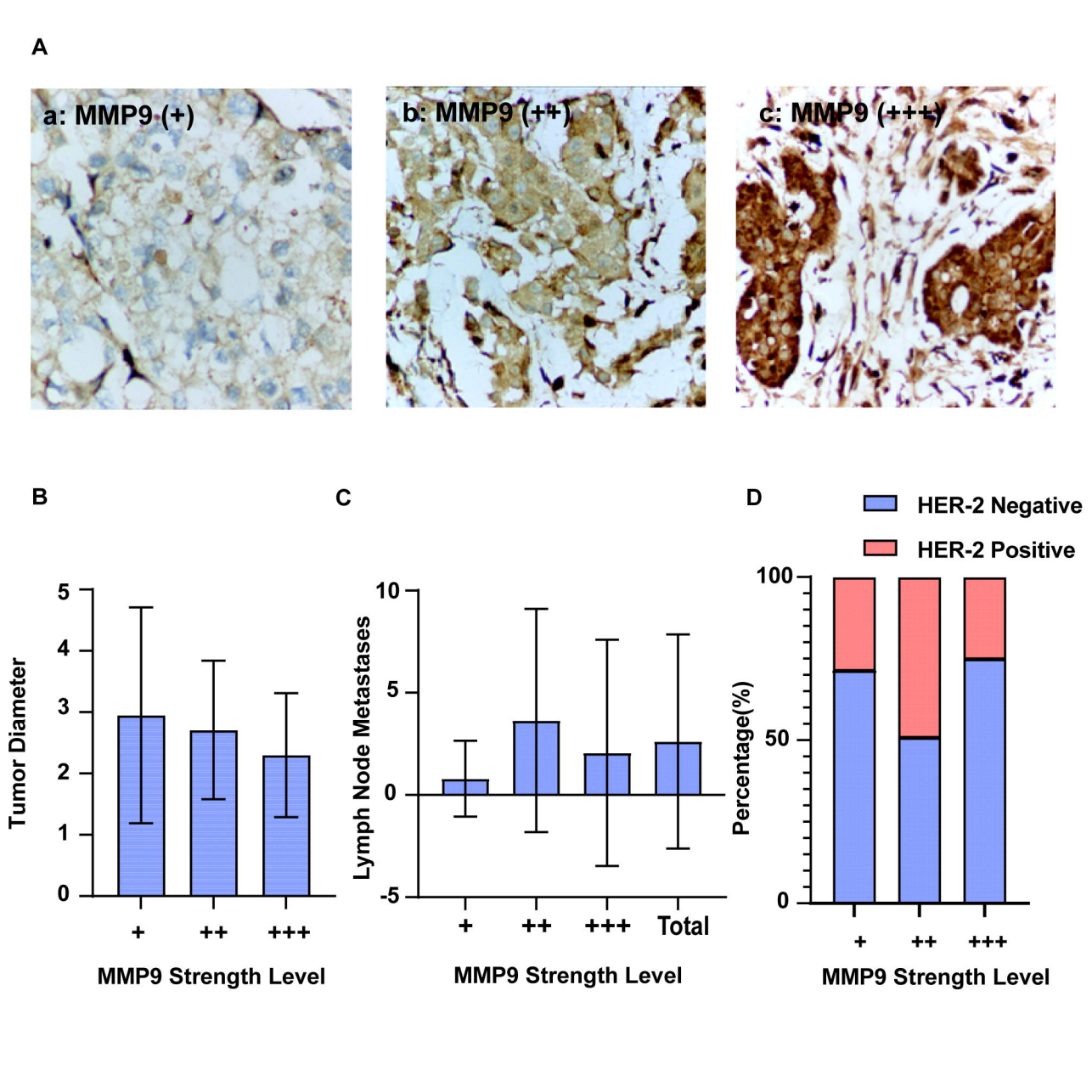
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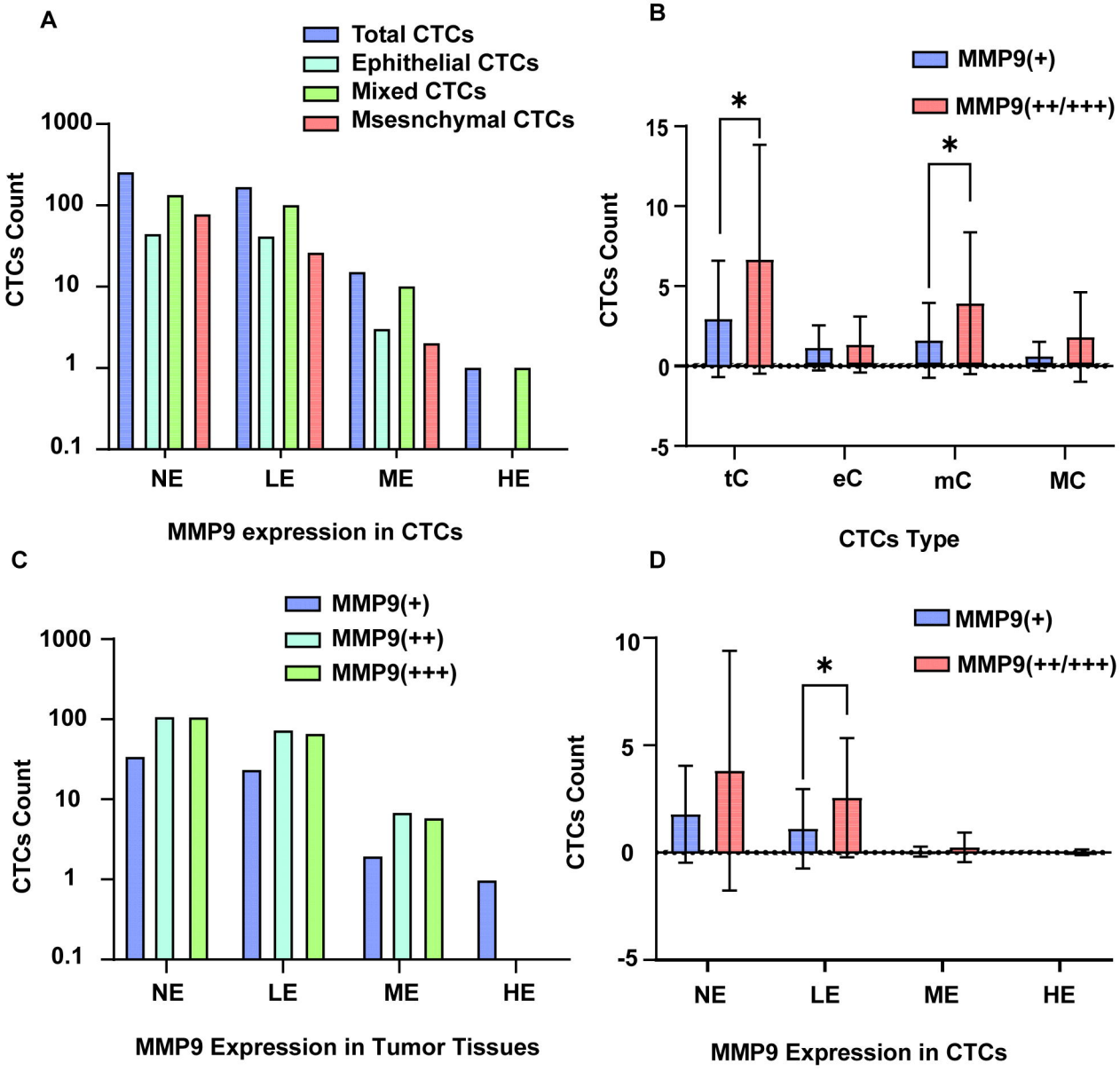
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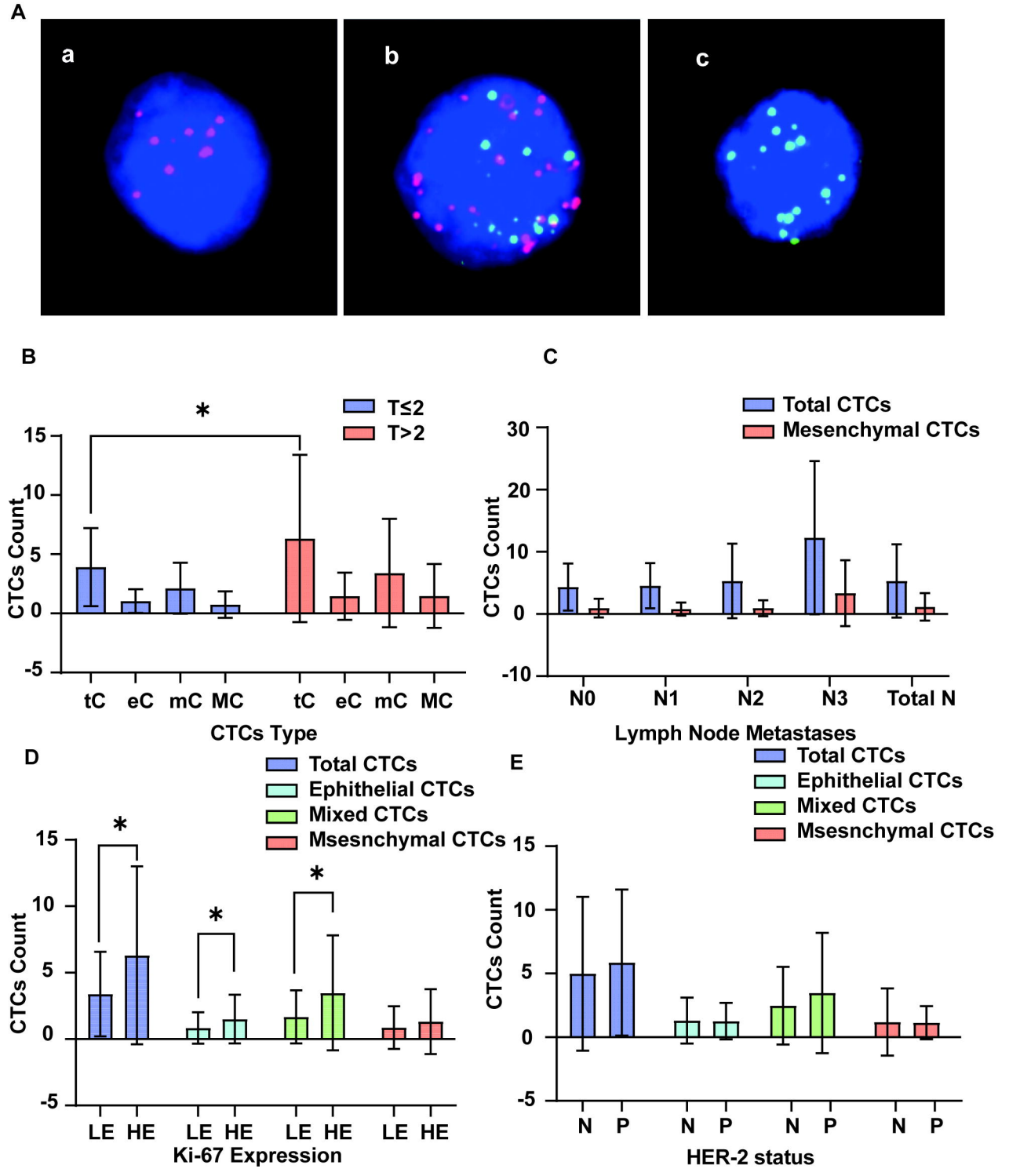
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Ki-67 Expression
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HER-2 status