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Jagged1 modulated tumor-associated macrophage differentiation predicts poor prognosis in patients with invasive micropapillary carcinoma of the breast

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Abstract

Objectives: Invasive micropapillary carcinoma of the breast (IMPC) constitutes a unique and aggressive subtype of breast cancer. We aimed to evaluate the prognostic significance of the Jagged1 (a ligand of the Notch pathway) expression, and infiltration density of tumor-associated macrophages (TAMs) in patients with IMPC.

Methods: Jagged1 expression and CD163+, CD68+ macrophage infiltration were evaluated by immunohistochemistry in 222 tumor samples, and the clinical significance was analyzed. mRNA level of Jagged1 was analyzed by real time PCR in tumor tissues.

Results: The IMPC patients showed larger tumor size, more lymphatic invasion, higher expression levels of estrogen receptor (ER), increased Ki67 index, higher Jagged1 protein level, and denser infiltration of CD163+ macrophages compared to patients with invasive breast ductal carcinoma. In the IMPC cohort, positive Jagged1 expression was related to aggressive features including large tumor size, lymphatic invasion, and Ki67 overexpression. Statistical significance was found between CD163+ macrophage infiltration and Jagged1 expression levels. Cox regression analysis revealed that ER negativity, positive Jagged1 expression, and a high degree of CD163+ macrophage infiltration were independent prognostic factors for disease-free survival, and positive Jagged1 expression was an independent prognostic factor for overall survival. The level of Jagged1 mRNA was higher in tumor tissues of patients with IMPC.

Conclusion: Jagged1, by modulating TAMs infiltration, is associated with a less favorable prognosis for patients with IMPC. Our results have important implications for therapies targeting Jagged1-Notch signaling and re-educating TAMs polarization for patients with IMPC.

Abbreviations: ER = estrogen receptor, HR = hazard ratio, IDC = invasive ductal carcinoma of the breast, IMPC = invasive micropapillary carcinoma of the breast, TAM = tumor-associated macrophage.

Keywords: CD163, invasive micropapillary carcinoma of the breast, Jagged1, prognosis, tumor-associated macrophages

1. Introduction

Invasive micropapillary carcinoma of the breast (IMPC) constitutes a rare and aggressive subtype of breast cancer, with the incidence rate ranging from 1.0% to 8.4%.[1–4] Previous studies have illustrated that IMPC exhibits a high frequency of peritumorallympho-vascular invasion, lymph node metastases, and increased local recurrence, and has a short duration of disease-free survival.[2,5] Estrogen receptor (ER) positivity is reportedly higher in IMPC cases, while it is distinct from ER-positive invasive ductal carcinoma of the breast (IDC) owing to its high proliferation rate.[5,6] At the genomic level, previous studies revealed a high prevalence of mutations and cytogenetic aberrations, including those in chromosome 8, compared with the control groups.[7,8] Morphologically, this carcinoma is characterized by breast cancer cells organized in pseudopapillary clusters separated from the surrounding loose fibrocollagenous-stroma by clear space.[9] Components in the extracellular matrix were reported to contribute to the aggressive behavior of IMPC.[10] Metastasis requires a permissive microenvironment and a variety of interactions between the tumor cells and the surrounding extracellular matrix.[11,12] Therefore, the elucidation of the underlying mechanisms and functions of microenvironment components in IMPC is significant.

Tumor-associated macrophages (TAMs) are one of the main populations of infiltrating immune cells in the tumor microenvironment and are critical mediators of tumor growth.[13–15] Currently, the most widely accepted classification of macrophage

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HL and JW contributed equally to this work and should be considered as co-first authors.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The study was approved by the Ethical Committee of Tumor Hospital of Harbin Medical University. Informed consent was obtained from all individual participants included in the study.

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polarization is classically activated macrophages (M1) and alternatively activated macrophages (M2).[14] According to numerous studies, TAMs share many properties of M2 macrophages, including expressing the scavenging receptor, CD163.[16,17] Medrek *et al* have reported that CD163+ macrophages in the breast tumor stroma are positively correlated with adverse clinicopathological features, while CD68+, the pan-macrophage markers are not.[18] In a previous, our research team identified a significant correlation between CD163+ macrophages and reduced progression-free survival in postmenopausal patients with breast cancer who received antiendocrine therapy.[19]

TAMs can be modulated by various cytokines and signaling pathways. Their differentiation is dependent on recombinant recognition sequence binding protein (RBPJ), the transcriptional regulator of Notch signaling, and they were observed to display a gene expression signature associated with the Notch pathway in a mouse mammary model.[20] Furthermore, an in vivo study revealed that mice deficient in RBPJ in the myeloid compartment presented with an impaired M2 polarization phenotype and that RBPJ is involved in the mediation of expression of a subset of M2 genes.[21,22] Notch signaling is an evolutionarily conserved pathway involved in tissue development and homeostasis.[23] In mammals, 4 distinct Notch receptors have been identified, Notch-1, -2, -3, and -4, which are bound by 5 ligands of the Jagged family and Delta-like family (Jagged-1, -2, and Delta-like ligand-1, -3, and -4).[24] RBP-J (also known as CSL or CBF1) is a key DNA-binding protein in the Notch signaling pathway, located in Notch-induced gene promoters.[22] High levels of Jagged1 mRNA and protein in breast cancer are responsible for the more aggressive features of the disease and may relate to tumor cell dissemination and metastatic progression.[25] Moreover, Notch activation was detectably upregulated in M2 polarized macrophages, and the blockage of Notch signaling by a γ-secretase inhibitor could reverse M2 differentiation in liver tissues in a murine model of Schistosoma japonica infection.[26]

However, it is unclear whether the Jagged1-Notch signaling pathway and TAM polarization in tumor stroma are involved in the adverse outcomes of patients with IMPC. In this study, we evaluated the clinical and prognostic value of Jagged1 expression and TAM infiltration in patients with IMPC.

2. Materials and methods

2.1. Patient background and eligibility

In total, 222 patients with breast cancer (102 IMPC and 120 IDC cases) were included in our study, and the clinicopathological and molecular parameters of the patients were documented. Primary tumor sections were obtained from the patients for immunohistochemistry. The patients had undergone mammectomy at the Department of Mammary Surgery of the Tumor Hospital of Harbin Medical University between May 2009 and March 2013. Patients with distant metastasis or those who had received neoadjuvantendocrine therapy or chemotherapy before mammectomy were excluded from the study. The date of surgery served as the beginning of the follow-up, which was completed in April 2016. The median follow-up time was 39 months (ranging between 9 and 79 months). This study was approved by the Ethics Committee of Tumor Hospital of Harbin Medical University, and written informed consent was signed by each participating patient before enrollment in our study.

2.2. Immunohistochemical staining

The specimens were formaldehyde-fixed and paraffin-embedded (FFPE) after the surgery and stored at 4 °C. Tissue slices of 4-μm thickness were used for immunohistochemical staining. Briefly, the slices were deparaffinized in xylene and rehydrated in series of alcohol gradients. A heat-mediated antigen retrieval step was performed using a pressure cooker in sodium citrate buffer (10 mM sodium citrate; 0.05% Tween 20; pH 6.0) before endogenous peroxidase activity was blocked with 3% H_2O_2. The sections were incubated overnight with primary antibodies diluted in bovine serum albumin, including CD163 mouse antihuman monoclonal antibody (TA506391, diluted 1:500, OriGene, Rockville, MD), CD68 mouse antihuman monoclonal antibody (ab31630, diluted 1:100, Abcam, Cambridge, UK), and Jagged1 rabbit antihuman polyclonal antibody (ab109536, dilution 1:200, Abcam, Cambridge, UK), in a humidified chamber at 4 °C. Subsequently, secondary antibody was added, and the samples were incubated at room temperature. The specimens were stained with 3,3′-diaminobenzidine for the visualization of results. The negative-staining control was generated by replacing the primary antibody with phosphate-buffered saline plus 1% bovine serum albumin.

2.3. Immunohistochemical staining evaluation

The immunohistochemical results were independently evaluated by 2 pathologists, who were blind to the patient characteristics. Each sample was observed under high power magnification (100×). Samples with >10% staining of macrophages in the tumor stroma were regarded as CD163/CD68 positive. The sections were scored semiquantitatively for Jagged1 staining with the following criteria: percentages of positive staining were classified as 0 (<10%), 1 (10%–30%), 2 (30%–50%), and 3 (>50%), and staining intensities were classified as 0 (absent), 1 (weak), 2 (moderate), and 3 (dense). Samples with a total score (intensity score multiplied by percentage score) >2 were classified as Jagged1-positive, and the remaining samples were classified as Jagged1-negative.

2.4. Total RNA extraction

Six to 8 sections (10 μm in thickness) of each FFPE tissue were used for total RNA extraction. The areas for sampling were marked on 10 μm-thick sections by pathologists to achieve high tumor content (>90%). Total RNA was isolated using the Total Nucleic Acid Isolation Kit (Ambion, Carlsbad, CA) following the manufacturers' protocols. All RNAs were tested using the Smart Spec Plus Spectrophotometer (Bio-rad, Hercules, CA).

2.5. Real time PCR for Jagged1 mRNA

Total RNA from FFPE tissues was reverse transcribed using the Prime Script RT reagent Kit with gDNA Eraser (TaKaRa, Kusatsu city, Japan), and cDNA was used to amplify Jagged1, with β-actin as an internal control. Real time PCR was performed using the SYBR Green Master kit (Roche, Basel, Switzerland) and the Applied Biosystems PRISM 7500 Fast Real-time PCR system (Thermo Fisher, Waltham, MA), with GAPDH as a reference control. Primers used in the PCR analyses are shown below. The forward sequence for the Jagged1 gene was 5′-CTATGATGAGGGGGATGCT-3′, and the reverse sequence was 5′-CGTCCATTCAGGCACTGG-3′. The forward sequence for the

Notch1 gene was 5′-CACTGTGGGCGGGTCC-3′, and the reverse sequence was 5′-GTTGTATTGGTTCGGCACCAT-3′.[27]

2.6. Statistical analysis

All the statistical analyses and graphing were performed using SPSS statistical software (version 20.0, IBM SPSS, Armonk, NY). Pearson chi-square test was used to analyze differences between the clinicopathological features in the IMPC and IDC cohorts, and the clinical significance of CD163+, CD68+ macrophage infiltrating density and Jagged1 expression. Kaplan–Meier analysis and the log-rank test were used to estimate survival curves and evaluate distributions. The Cox proportional hazards regression models of factors related to survival were used to calculate hazard ratios and to identify the factors that affect survival in both uni- and multivariate analysis. All statistical tests were 2-sided and $P < .05$ was considered significant.

3. Results

3.1. Comparison of clinicopathological features of patients with IMPC and IDC

The patients' clinicopathological features are summarized in Table 1. The tumor size in IMPC patients was larger compared with that in the IDC patients ($P = .012$). Patients with IMPC had a higher degree of lymphatic invasion and ER positivity compared to patients with IDC ($P < .001$, $P = .047$, respectively). There was a trend toward a higher Ki67 index in patients with IMPC than in patients with IDC ($P = .011$). Jagged1 expression and CD163+ macrophage infiltration were higher in the tissues of patients with IMPC than in patients with IDC ($P = .043$, $P = .003$, respectively; Table 1).

3.2. Clinical significance of CD163+, CD68+ macrophage infiltration and Jagged1 expression in patients with IMPC

Positive staining of Jagged1 was observed in the membranes of tumor cells (Fig. 1). CD163+, CD68+ macrophages were mainly

Table 1

Clinicopathological features of IMPC and IDC patients.

	IMPC patients (n, %)	IDC patients (n, %)	*P*
Age, y (mean/median/range)	46/47/26–65	45/46/33–66	
BMI, kg/m^2			
<24	56 (37.25)	73 (61.67)	.414
≥24	46 (62.74)	47 (38.33)	
Tumor size, cm			
<2	30 (29.41)	39 (32.50)	.012
2–5	31 (30.39)	54 (45.00)	
>5	41 (40.20)	27 (22.50)	
Lymphatic invasion			
N0-N1	31 (30.39)	76 (63.33)	<.001
N2-N3	71 (69.61)	44 (36.67)	
ER status			
negative	28 (27.45)	49 (40.83)	.047
positive	74 (72.55)	71 (59.17)	
PR status			
negative	49 (48.04)	42 (41.18)	.056
positive	53 (51.96)	78 (76.47)	
Her-2 status			
Negative	77 (75.49)	89 (74.17)	.877
Positive	25 (24.51)	31 (25.83)	
Ki 67, %			
<14	47 (46.08)	76 (63.33)	.011
≥14	55 (53.92)	44 (36.67)	
Jagged1 expression level			
Negative	37 (36.27)	68 (56.67)	.003
Positive	65 (63.73)	52 (43.33)	
CD163+ macrophage infiltration			
Negative	40 (39.22)	64 (53.33)	.043
Positive	62 (60.78)	56 (46.67)	
CD68+ macrophage infiltration			
Negative	43 (42.16)	52 (43.33)	.892
Positive	59 (57.84)	68 (56.67)	

BMI = body mass index, ER = estrogen receptor, Her-2 = human epidermal receptor 2, IDC = invasive ductal carcinoma of the breast, IMPC = invasive micropapillary carcinoma of the breast, PR = progestogen receptor.

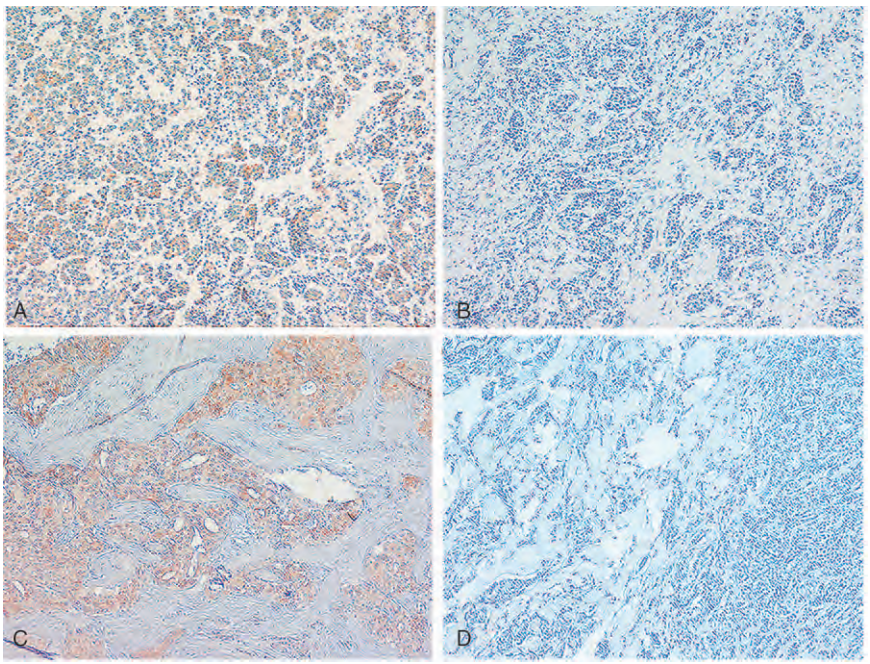

Figure 1. Immunohistochemistry was performed to detect Jagged1 protein level in tumor tissues. The staining results were observed under a high power lens (×100). (A) Jagged1 positive staining in IMPC tissues, (B) Jagged1 negative staining in IMPC tissues, (C) Jagged1 positive staining in IDC tissues, and (D) Jagged1 negative staining in IMPC tissues. IDC = invasive ductal carcinoma of the breast, IMPC = invasive micropapillary carcinoma of the breast.

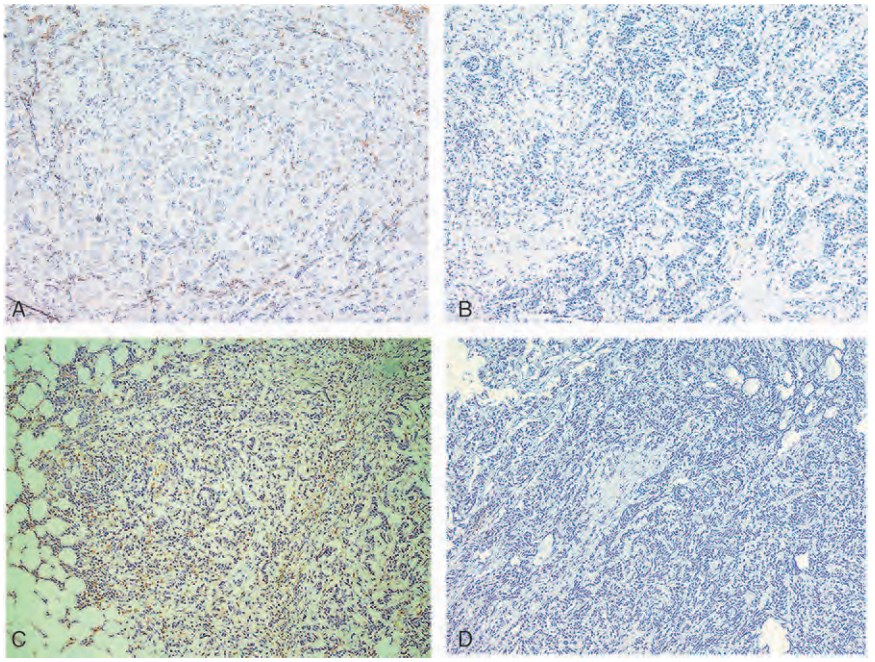

Figure 2. Immunohistochemical staining of stromal CD163+ macrophages in tumor tissues (×100). (A) High infiltration level of stromal CD163+ macrophages in IMPC tissues, (B) stromal CD163+ macrophages negative staining in IMPC tissue, (C) high infiltration level of stromal CD163+ macrophages in IDC tissues, and (D) stromal CD163+ macrophages negative staining in IDC tissues. IDC = invasive ductal carcinoma of the breast, IMPC = invasive micropapillary carcinoma of the breast.

distributed along the invasive margin of the tumor (Figs. 2 and 3). We evaluated the association between CD163+/CD68+ macrophage infiltration or Jagged1 expression and clinicopathological parameters in the IMPC patient cohort. High infiltration of CD163+ macrophages was related to lymphatic invasion ($P = .015$). A statistically significantly higher CD68+ macrophage infiltration density was identified in larger tumors ($P = .019$). Jagged1 expression was significantly correlated with tumor size, lymphatic invasion number, and Ki-67 expression level ($P = .043$, .014, and .022, respectively) as shown in Table 2. Additionally,

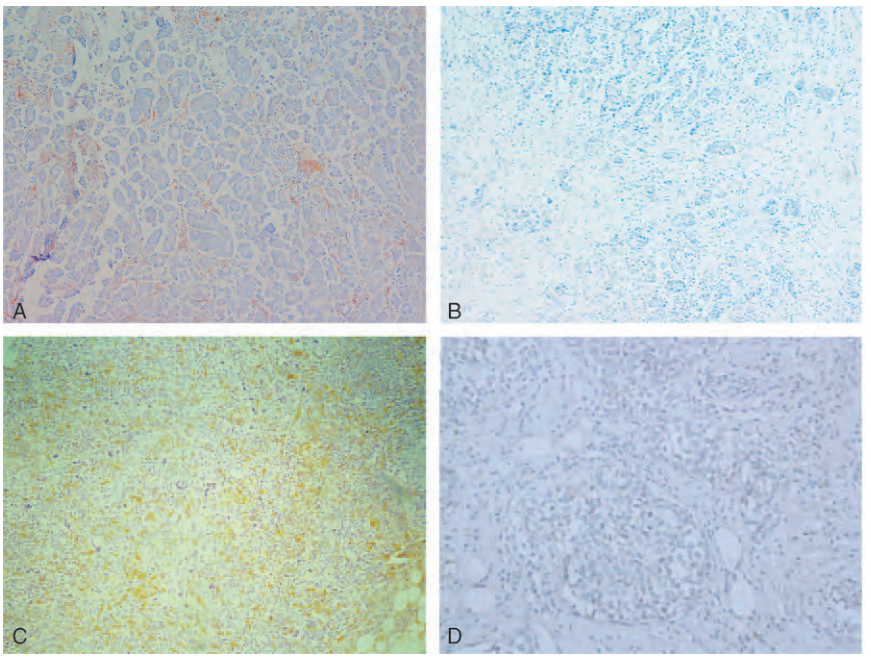

Figure 3. Immunohistochemical staining of stromal CD68+ macrophages in tumor tissues (×100). (A) High infiltration level of stromal CD68+ macrophages in IMPC tissues, (B) stromal CD68+ macrophages negative staining in IMPC tissue, (C) high infiltration level of stromal CD68+ macrophages in IDC tissues, and (D) stromal CD68+ macrophages negative staining in IDC tissues. IDC = invasive ductal carcinoma of the breast, IMPC = invasive micropapillary carcinoma of the breast.

Table 2

The CD163+, CD68+ macrophage infiltration and expression of Jagged1 in 102 IMPC patients and their association with clinicopathological features.

Clinicopathological feature	CD163+ macrophage infiltration (n, %)		P	CD68+macrophage infiltration (n, %)		P	Jagged1 expression (n,%)		P
	High	Low		High	Low		Positive	Negative	
BMI (Kg/m^2) <24	35 (34.31)	21 (20.59)	.839	40 (39.22)	36 (35.29)	.264	39 (38.24)	17 (16.67)	.215
≥24	27 (26.47)	19 (18.62)		19 (18.63)	27 (26.47)		26 (25.49)	20 (19.61)	
Tumor size (cm)<2	14 (13.73)	16 (15.69)	.073	11 (10.78)	19 (18.63)	.019	14 (10.78)	16 (12.75)	.043
2–5	18 (17.65)	13 (12.75)		20 (19.61)	11 (10.78)		20 (32.35)	11 (13.73)	
>5	30 (29.41)	11 (10.78)		28 (27.45)	13 (12.75)		31 (20.59)	10 (9.80)	
Lymphatic invasion									
N0N1	13 (12.75)	18 (17.65)	.015	15 (14.71)	16 (15.69)	.276	14 (13.73)	17 (16.67)	.014
N2N3	49 (48.04)	22 (21.57)		44 (43.14)	27 (26.47)		51 (50.00)	20 (19.61)	
ER status negative	15 (14.71)	13 (12.75)	.373	13 (12.75)	15 (14.71)	.181	16 (15.69)	12 (11.76)	.490
positive	47 (46.08)	27 (26.47)		46 (45.10)	28 (27.45)		49 (45.10)	25 (27.45)	
PR status negative	25 (13.72)	24 (16.67)	.068	26 (25.49)	23 (22.55)	.423	34 (33.33)	15 (14.71)	.305
positive	37 (34.31)	16 (35.29)		33 (32.35)	20 (19.61)		31 (30.39)	22 (21.57)	
Her2 status negative	45 (44.12)	32 (31.37)	.483	47 (46.08)	30 (29.41)	.315	46 (45.10)	31 (30.39)	.157
positive	17 (16.67)	8 (7.84)		12 (11.76)	13 (12.75)		20 (19.61)	6 (5.88)	
Ki67 (%) <14%	25 (24.51)	22 (21.57)	.160	26 (26.47)	21 (19.61)	.690	24 (23.53)	23 (22.55)	.022
≥14%	37 (36.27)	18 (17.65)		33 (31.37)	22 (22.55)		41 (40.20)	14 (13.73)	

ER = estrogen receptor, Her-2 = human epidermal receptor 2, IDC = invasive ductal carcinoma of the breast, IMPC = Invasive micropapillary carcinoma of the breast, PR = progestogen receptor.

Table 3

CD163+ macrophage infiltration density was positively correlated with Jagged1 expression in 102 IMPC patients.

	CD163 high (n,%)	CD163 low (n,%)	P	R
Jagged1+	49 (48.04)	16 (15.69)	<.001	0.396
Jagged1-	13 (12.75)	24 (23.53)		

IMPC = Invasive micropapillary carcinoma of the breast.

Table 4

The correlation of CD68+ macrophage infiltration density with Jagged1 expression in 102IMPC patients.

	CD68 high (n,%)	CD68low (n,%)	P	R
Jagged1+	39 (38.24)	26 (25.49)	.677	0.058
Jagged1-	20 (19.61)	17 (16.67)		

IMPC = Invasive micropapillary carcinoma of the breast.

Table 5

Univariate and multivariate analysis of clinicopathological features associated with survival and recurrence in IMPC patients.

Clinicopathological Features	Disease-free survival				Overall survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age (y) <50 vs ≥50	2.176 (0.947–1.998)	.067			1.367 (0.569–1.287)	.485		
BMI ≥24 vs. <24	1.838 (0.429–1.160)	.926			1.082 (0.406–2.103)	.924		
Tumor size (cm) 2–5vs <2	1.412 (0.470–1.638)	.539			1.085 (0.348–1.385)	.888		
>5vs <2	1.451 (0.557–1.778)	.446			1.065 (0.379–1.999)	.905		
Lymphatic invasion								
N2-N3vs N0-N1	1.147 (0.741–1.775)	.540			1.237 (0.800–1.912)	.339		
ER status								
negative vs positive	1.675 (0.457–2.781)	<.001	1.038 (0.492–1.863)	.003	1.071 (0.456–1.909)	.347		
PR status								
negative vs positive	1.229 (0.620–1.770)	.140			1.134 (0.685–1.436)	.331		
Her-2 status positive vs negative	1.613 (1.260–2.066)	<.001	0.651 (0.797–1.352)	.783	1.440 (1.137–1.825)	.003	1.155 (0.892–1.497)	.275
Ki 67 (%) ≥14 vs. <14	1.293 (0.457–2.297)	.325			1.066 (0.441–1.218)	.230		
Jagged1 expression								
positive vs negative	2.142 (1.681–2.729)	<.001	1.486 (1.084–2.037)	.014	1.523 (1.215–1.890)	.014	1.586 (1.066–2.360)	.023
CD163+macrophage infiltration								
high vs low	3.189 (2.341–4.343)	<.001	2.370 (1.602–3.505)	<.001	1.769 (1.387–2.256)	.011	1.060 (0.744–1.510)	.747
CD68+maccrophage infiltration								
high vs low	1.046 (0.542–1.215)	.536			1.030 (0.871–1.312)	.327		

CI = confidence interval, ER = estrogen receptor, HR = hazard ratio, Her-2 = human epidermal receptor 2, IDC = invasive ductal carcinoma of the breast, IMPC = invasive micropapillary carcinoma of the breast, PR = progestogen receptor.

Jagged1 expression in breast cancer tissues was positively correlated with CD163+ macrophage infiltration in the tumor stroma ($P < .001$; Table 3). However, no statistically significant association was found between Jagged1 expression in tumors and the presence of stromal CD68+ macrophages (Table 4).

3.3. Evaluation of independent risk factors for disease-free survival (DFS) and overall survival (OS) in patients with IMPC

As shown in Table 5, the prognostic value of each clinicopathological feature was evaluated in the univariate and multivariate analysis. In the univariate analysis, high infiltration of CD163+ macrophages, positive Jagged1 expression, and human epidermal growth factor 2 over-expression were risk factors for both OS and DFS ($P < .05$ for all). In the multivariate analysis, independent risk factors for DFS include positive Jagged1 expression, high CD163+ macrophage infiltration, and absent ER positivity ($P = .014$, $P < .001$, and $P = .003$, respectively), while Jagged1 expression was an independent risk factor for OS ($P = .023$) (Table 5).

3.4. Impact of CD163+ macrophage infiltration and Jagged1 protein expression on patient survival

The differences in survival between the IMPC and IDC cohorts were evaluated, and the patients with IMPC were found to have DFS and OS ($P = .001$, .008, respectively; Fig. 4A, B). We also

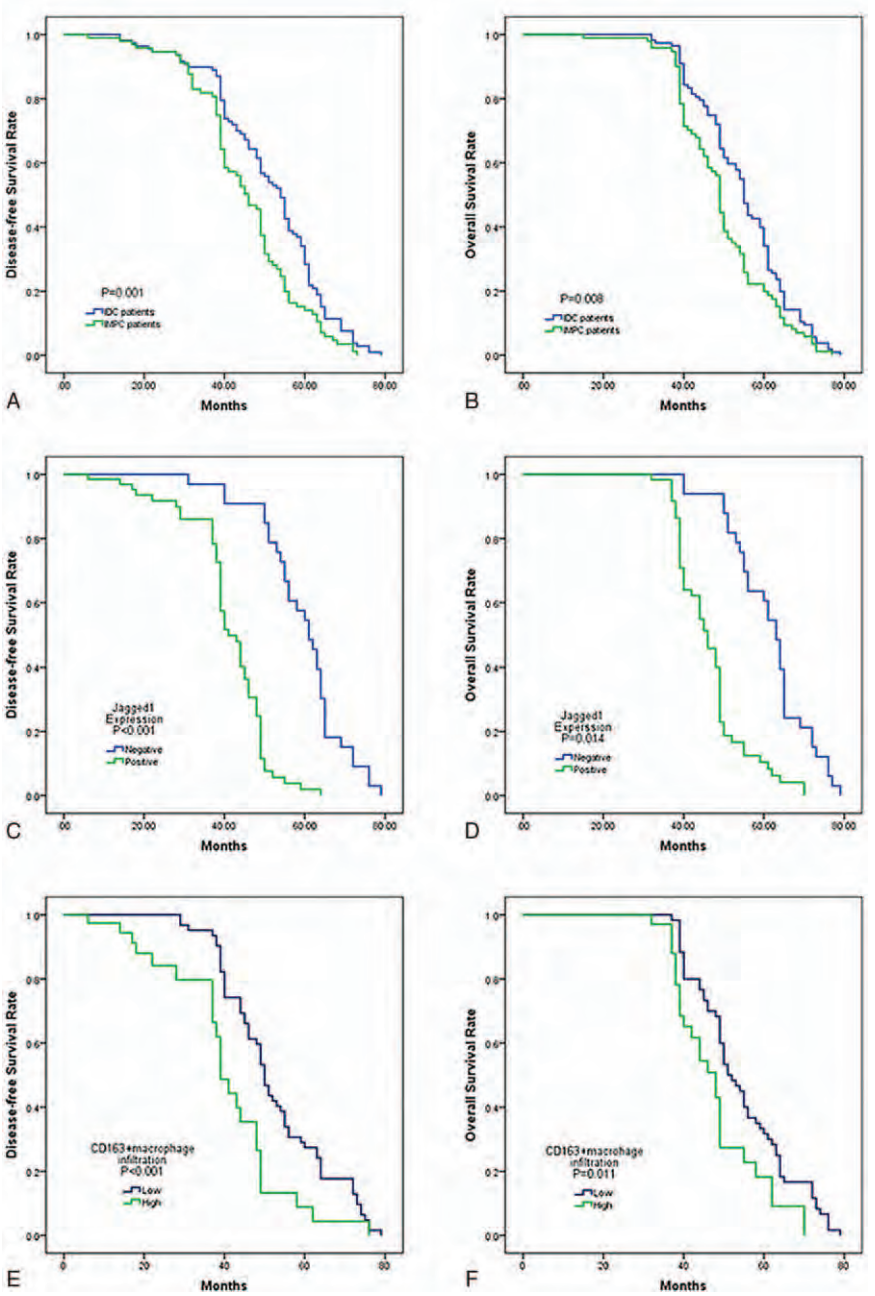

Figure 4. Kaplan–Meier analysis for DFS and OS. (A) Comparison of DFS between IMPC and IDC patients, (B) comparison of OS between IMPC and IDC patients, (C) predictive value of Jagged1 protein level on DFS in IMPC patients, (D) predictive value of Jagged1 protein level on OS in IMPC patients, (E) predictive value of stromal CD163+ macrophages on DFS in IMPC patients, and (F) predictive value of stromal CD163+ macrophages on OS in IMPC patients. DFS = disease-free survival, IDC = invasive ductal carcinoma of the breast, IMPC = invasive micropapillary carcinoma of the breast, OS = overall survival.

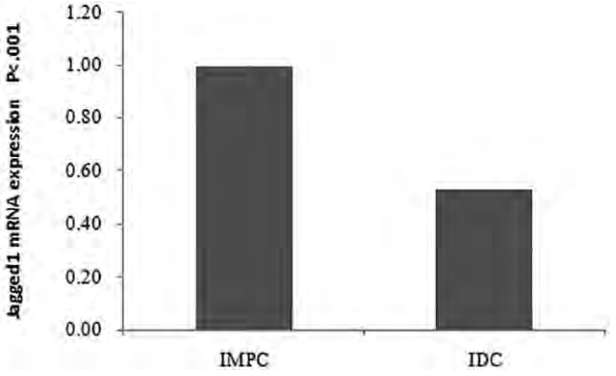

Figure 5. Comparison of mRNA levels of Jagged1 in patients with IMPC and IDC. IDC = invasive ductal carcinoma of the breast, IMPC = invasive micropapillary carcinoma of the breast.

analyzed the impact of CD163+ macrophage infiltration and Jagged1 expression on the DFS and OS of IMPC patients. Furthermore, the patients with IMPC with positive expression of Jagged1 suffered reduced DFS and OS ($P < .001$, $P = .014$, respectively; Fig. 4C, D). A high degree of CD163+ macrophage infiltration was negatively correlated with the DFS and OS of the patients with IMPC ($P < .001$, $P = .011$, respectively; Fig. 4E, F).

3.5. Comparison on Jagged1 mRNA level in tumor tissues of patients with IMPC and IDC

Real time PCR was applied to examine the mRNA level of Jagged1 in tumor tissues of patients with IMPC and IDC. The results showed that Jagged1 mRNA level was upregulated in samples of patients with IMPC (Fig. 5).

4. Discussion

IMPC of the breast is a morphologically distinct subtype of breast carcinoma. Since the recent recognition of IMPC in the mid-1990s, this disease has received increasing attention owing to the relatively advanced stages at diagnosis, lymph node involvement, and high incidence of local recurrence and distant metastasis.[4] Previous studies have revealed strong expression of E-cadherin on adjacent surfaces of IMPC tumor cell clusters, yet weak or negative expression on the outer surface of tumor cells toward stroma.[28] The adhesion between the tumor cell clusters and stroma appears loose, which results in the enhanced motility and invasiveness of the tumor cells.[6] Components in the microenvironment also contribute to the aggressiveness of IMPC. The absence of caveolin-1 expression in carcinoma-associated fibroblasts predicts poor outcomes for patients with IMPC. In the present study, we found a higher incidence of lymphatic invasion, ER positivity, and Ki67 over-expression in the IMPC cases, which is consistent with previous studies that demonstrated IMPC as luminal B subtype.[29]

TAMs are key orchestrators in the tumor microenvironment and directly impact angiogenesis, extracellular-matrix remodeling, tumor cell motility, and metastasis.[29] The increased infiltration of TAMs is correlated with resistance to therapy and unfavorable outcome in patients with breast cancer. TAMs have been characterized mainly as M2 macrophages. These macrophages express elevated levels of immunosuppressive cytokines, including arginase-1, mannose receptor, and transforming growth factor β.[17] Our previous study reported that stromal TAMs negatively influenced the infiltration of natural killer cells by producing growth-arrest specific protein 6 and could predict poor outcomes for patients with triple-negative breast cancer.[30] In our study, the high infiltration density of TAMs in the tumor stroma was also demonstrated to be associated with reduced survival in the patients with IMPC.

Jagged1 is the most important ligand of Notch signaling, which closely relates to development and metastasis in breast cancer.[27] The classic Jagged1–Notch interaction results in a cascade of proteolytic cleavages, leading to the transportation of the Notch intracellular domain into the nucleus and the activation of downstream transcription of target genes.[31] Jagged1 has been demonstrated to promote tumor growth by stimulating interleukin (IL)-6 release from osteoblasts and directly activated osteoclast differentiation, which consequently contributed to breast cancer bone metastasis.[32] Jagged1-mediated Notch signaling activation may induce the epithelial-to-mesenchymal transition via slug-induced suppression of E-cadherin.[27] In another study, it was found that Jagged1–Notch4-dependent cancer stem cell activity could induce antiestrogen resistance in breast cancer patients.[33] Furthermore, Jagged1 was demonstrated to be associated with the overexpression of proliferation marker Ki-67, and an assessment of Jagged1 protein status in circulating tumor cells may serve as a promising diagnostic strategy to predict the response to antitumor therapies in patients with breast cancer.[34] In the present study, we found Jagged1 protein level in breast cancer cells was positively correlated with unfavorable clinical features and was strongly associated with reduced DFS and OS in IMPC patients, a result that was in accordance with previous studies.[35] We also observed that the mRNA level was higher in tumor samples of patients with IMPC, which consistent with an early study suggesting that high level of Jagged1 mRNA predicts poor outcome in breast cancer patients.[25]

A considerable body of work implicates the Notch pathway as an important regulator of macrophage function. TAM differentiation was reported to depend on Notch signaling modulation. Toll-like receptor stimulation activates Notch signaling and regulates gene expression in activated macrophages. Notch-1 upregulation and signaling following macrophage activation modulate gene expression patterns that affect antigen-presenting capacity and cytotoxic activity. Furthermore, interferon regulator factor 8 and suppressor of cytokine signaling 3 have been identified as downstream targets of Notch signaling in the regulation of macrophage activation. In our study, Jagged1 expression in cancer cells was positively correlated with stromal TAM infiltration density in the IMPC patient cohort. The elevated TAM polarization may promote the release of various cytokines and chemokines by tumor cells, including IL10 and EGFR, and consequently contribute to the aggressive characteristics of IMPC. Therefore, a potential treatment approach may depend on the downregulation of the Jagged1–Notch pathway to suppress the potentially protumorigenic functions of TAMs in patients with IMPC.

In summary, we analyzed the prognostic significance of TAMs infiltration and Jagged1 expression in patients with IMPC and found that Jagged1 may correlate with poor patient prognosis via modulating TAMs differentiation. These aspects of Jagged1 functions require further verification in breast cancer cell lines and investigation in all pathological subtypes of breast cancers. Our results may provide information for developing new

prognostic biomarkers and improving the outcomes of patients with IMPC.

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