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Vimentin and tumor–stroma ratio for neoadjuvant chemoradiotherapy response prediction in locally advanced rectal cancer

Wenjing Tian[1,2] | Yuqin Yang[1,3,4] | Qi Qin[5] | Liguo Zhang[6] | Zheyan Wang[2] | Liqian Su[7] | Lirong Zeng[1] | Hui Chen[1] | Lingzhi Hu[1] | Jiawei Hong[6] | Ying Huang[1] | Qingling Zhang[3,4] | Hong Zhao[1,2]

[1]The Cancer Center of the Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China

[2]Guangdong Provincial Key Laboratory of Biomedical Imaging, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, China

[3]Department of Pathology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China

[4]Department of Pathology, School of Basic Medical Science, Southern Medical University, Guangzhou, China

[5]Department of Medical Oncology, The Second Affiliated Hospital of Hainan Medical University, Haikou, China

[6]Department of Thyroid & Breast Surgery, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, China

[7]Precision Medicine Center of Harbin Medical University Cancer Hospital, Harbin, China

Correspondence
Hong Zhao and Ying Huang, No. 56 Meihua East Road, Xiangzhou District, Zhuhai, China.
Email: zhaoh59@mail.sysu.edu.cn; 5393288@qq.com

Qingling Zhang, No. 106 Zhongshan er Road, Yuexiu District, Guangzhou, China.
Email: zhangqingling@gdph.org.cn

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Abstract

Vimentin expression in tumor tissues and the tumor–stroma ratio (TSR) have been demonstrated as strong prognostic factors for cancer patients, but whether they are predictive markers of neoadjuvant chemoradiotherapy (nCRT) outcome in locally advanced rectal cancer (LARC) patients is poorly understood. This study aimed to explore the predictive significance of vimentin and TSR combined for nCRT response in LARC patients. Imaging mass cytometry (IMC) was performed to determine the association of vimentin and TSR with nCRT response in six LARC patients [three achieved pathological complete response (pCR), three did not]. Immunohistochemistry (IHC) for vimentin and TSR on biopsy tissues before nCRT and logistic regression analysis were performed to further evaluate their predictive value for treatment responses in a larger patient cohort. A trend of decreased vimentin expression and increased TSR in the pCR group was revealed by IMC. In the validation group, vimentin [odds ratio (OR) 0.260, 95% confidence interval (CI) 0.102–0.602, $p = 0.002$] and TSR (OR 4.971, 95% CI 1.933–15.431, $p = 0.002$) were associated with pCR by univariate analysis. Patients in the vimentin-low/TSR-low or vimentin-high/TSR-high (OR 5.211, 95% CI

Abbreviations: 3D-CRT, three-dimensional conformal radiotherapy; BMI, body mass index; CA 19–9, carbohydrate antigen 19–9; CEA, carcinoembryonic antigen; cN stage, clinical N stage; cT stage, clinical T stage; DTAV, distance from anal verge; EMT, epithelial-to-mesenchymal transition; HB, hemoglobin; IHC, immunohistochemistry; IMC, imaging mass cytometry; IMRT, intensity-modulated radiation therapy; LARC, locally advanced rectal cancer; nCRT, neoadjuvant chemoradiotherapy; pCR, pathological complete response; TME, tumor microenvironment; TSR, tumor–stroma ratio.

Wenjing Tian, Yuqin Yang, and Qi Qin contributed equally to this work and should be considered co-first authors

1.248–35.582, $p = 0.042$) and vimentin-low/TSR-high groups (OR 11.846, 95% CI 3.197–77.079, $p = 0.001$) had significantly higher odds of pCR. By multivariate analysis, only the combination of vimentin and TSR was an independent predictor for nCRT response (OR 9.324, 95% CI 2.290–63.623, $p = 0.006$). Our study suggested that the combined assessment of vimentin and TSR can provide additive significance and may be a promising indicator of nCRT response in LARC patients.

KEYWORDS
locally advanced rectal cancer, neoadjuvant chemoradiotherapy, pathological complete response, tumor–stroma ratio, vimentin

1 | INTRODUCTION

Neoadjuvant chemoradiotherapy, followed by total mesorectal excision, has become the standard therapeutic schedule in patients with LARC.[1] However, among LARC patients treated with this regimen, the response rates vary immensely.[2] Pathological complete response is only achieved in 10%–20% of patients with LARC who receive nCRT, while the majority of patients experience mild to moderate tumor regression or even progression following nCRT.[2,3] This heterogeneity among LARC patients has aroused great interest in exploring predictive indicators, as these could aid in clinical decision-making.

Epithelial-to-mesenchymal transition is acknowledged to play an important role in progression and therapy resistance in cancers.[4–6] Acknowledged as a canonical biomarker of EMT, vimentin mediates multiple signaling pathways during this process and is involved in cancer cell adhesion, mitosis, and trafficking.[7–9] The role of vimentin in therapeutic responses has also been investigated in cancers, however paradoxical results were obtained. Hu *et al.* indicated that vimentin is highly expressed in gefitinib-resistant non–small-cell lung cancer cells,[10] while Kanakkanthata et al.[11] found that ovarian cancer cells acquired resistance to peloruside A and laulimalide with downregulation of vimentin. The conflict between different studies may indicate the insufficiency in the predictive capability of vimentin alone, and more factors should be incorporated to enhance the discriminative power.

The TME provides another opportunity to identify markers to predict therapy efficacy.[12,13] Stromal cells within the TME have been demonstrated to have crucial roles in tumorigenesis, cancer progression, and metastasis.[14] Moreover, the TSR, which assesses the extent of stromal proliferation within the borders of the primary tumor, was developed as a prognostic tool.[15] After first indicating its high prognostic value in colorectal cancer, the TSR has been validated in several types of tumors.[15–18] However, the therapeutic predictive value of the TSR in LARC patients, especially those who receive nCRT, is still poorly understood. Considering that both the tumor cells themselves and the TME mediate the treatment efficacy and the insufficient predictive power of a single marker, taking into account both vimentin and TSR, markers from tumor cells, and the TME, respectively, may provide additional predictive effects.

Therefore, in the current study, we were the first to explore whether the combination of TSR and vimentin in pre-nCRT biopsy specimens can provide predictive value for treatment response in LARC patients, hoping to allow more rational therapeutic strategies to be developed in the future.

2 | MATERIALS AND METHODS

2.1 | Patients

In total, 159 patients with LARC (six assigned to the discovery group and 153 assigned to the validation group) who received nCRT between December 2011 and September 2017 at Harbin Medical University Cancer Hospital and Fifth Affiliated Hospital of Sun Yat-sen University were enrolled in this retrospective study. The inclusion criteria were as follows: (1) pathological confirmation of LARC and complete records of medical information; (2) stage II/III disease in accordance with the 8th edition of the American Joint Committee on Cancer (AJCC) Staging Manual; (3) no history of other malignant tumors; and (4) no history of pelvic surgery, radiotherapy, or systemic chemotherapy.

The Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University and Harbin Medical University Cancer Hospital assessed and approved this study, and informed consent was required for all participants.

2.2 | Neoadjuvant chemoradiotherapy treatment

All patients underwent nCRT followed by total mesorectal excision. The neoadjuvant radiotherapy regimen consisted of IMRT and 3D-CRT. The radiation dose of the planning target volume (PTV) of clinical target volume (CTV) was 45 Gy with 25 fractions. The radiation dose of the PTV of gross tumor target volume (GTV) was 50 Gy in 25 fractions with IMRT or 50.4 Gy in 28 fractions with 3D-CRT. The chemotherapeutic regimens included capecitabine, XELIRI, CapeOX, 5-FU, FOLFOX4, and mFOLFOX6, and was prescribed at the discretion of the physician. Capecitabine- or 5-FU-based neoadjuvant chemotherapy was initiated on the first day of radiotherapy.

TABLE 1 Clinicopathological parameters

Variables	Discovery group *N* = 6	Validation group *N* = 153
Age, median (min, max)	55 (46,61)	57 (27,76)
Gender, *N* (%)		
Male	4 (66.7)	108 (70.6)
Female	2 (33.3)	45 (29.4)
BMI (kg/m^2), *N* (%)		
<25	5 (83.3)	123 (80.4)
≥25	1 (16.7)	30 (19.6)
cT stage, *N* (%)		
cT3	4 (66.7)	105 (68.6)
cT4	2 (33.3)	48 (31.4)
cN stage, *N* (%)		
cN0	2 (33.3)	57 (37.3)
cN+	4 (66.7)	96 (62.7)
Tumor length (cm), *N* (%)		
<4	3 (50.0)	73 (47.7)
≥4	3 (50.0)	80 (52.3)
DTAV (cm), *N* (%)		
<5	3 (50.0)	54 (35.3)
≥5	3 (50.0)	99 (64.7)
Differentiation, *N* (%)		
Poor/Moderate	4 (66.7)	93 (60.8)
Well	2 (33.3)	60 (39.2)
Histological type, *N* (%)		
Ulcerative	4 (66.7)	81 (52.9)
Other	2 (33.3)	72 (47.1)
CEA (ng/ml), *N* (%)		
<5	4 (66.7)	108 (70.6)
≥5	2 (33.3)	45 (29.4)
CA 19–9 (ng/ml), *N* (%)		
<39	6 (100.0)	145 (94.8)
≥39	0 (0.0)	8 (5.2)
HB (g/L), *N* (%)		
<90	0 (0.0)	19 (12.4)
90–120 (male)/110 (female)	3 (50.0)	84 (54.9)
≥120 (male)/110 (female)	3 (50.0)	50 (32.7)
Neo-chemo regime, *N* (%)		
Capecitabine based	4 (66.7)	72 (47.1)
5-Fluorouracil based	2 (33.3)	81 (52.9)
Neo-chemo cycles, *N* (%)		
1–2	0 (0.0)	27 (17.6)
3–4	5 (83.3)	112 (73.2)
5–6	1 (16.7)	14 (9.2)
Neo-radio technique, *N* (%)		
IMRT	6 (100.0)	136 (88.9)
3D-CRT	0 (0.0)	17 (11.1)
Neo-radio dose (Gy), *N* (%)		
<50	1 (16.7)	28 (18.3)
≥50	5 (83.3)	125 (81.7)

Abbreviations: 3D-CRT, three-dimensional conformal radiotherapy; BMI, body mass index; CA 19–9, carbohydrate antigen 19–9; CEA, carcinoembryonic antigen; cT stage, clinical T stage; cN stage, clinical N stage; DTAV, distance from anal verge; HB, hemoglobin; IMRT, intensity-modulated radiation therapy.

2.3 | Imaging mass cytometry

Formalin-fixed paraffin-embedded sections were dewaxed, rehydrated, and then subjected to antigen retrieval with Tris-EDTA buffer. After cooling to room temperature, the tissues were blocked with 3% BSA. Then, antibodies, including anti-vimentin (1:300, 3143027D, Fluidigm) and anti-E-cadherin antibodies (1:400, 3158029D, Fluidigm), were prepared at the same time. The slides were incubated with the antibody cocktail at 4°C overnight. Each slide was washed, and the DNA was labeled with Intercalator-Ir the next day. Before acquiring IMC, the slides were rinsed with ddH_2O and then air dried. Reagents were purchased from Fluidigm, and all steps were performed in accordance with the instructions from the manufacturer. Areas of 500×500 μm were selected and, for each slide, three regions of interest were chosen.

2.4 | Immunohistochemistry

Tissue sections were dewaxed, rehydrated, and then boiled in Tris-EDTA buffer. After incubation with 0.3% hydrogen peroxide, the slides were blocked with 3% BSA. Anti-vimentin antibody (1:100 dilution, AF7013, Affinity) was used to incubate the slides at 4°C overnight, and then the slides were labeled with an HRP-conjugated secondary antibody the next day. Positive staining was observed with diaminobenzene substrate solution and then counterstained with hematoxylin.

Two pathologists who were unaware of the clinical information carried out the evaluation of the sections. A consensus was reached through discussion if there were differences in scoring between the two pathologists. Vimentin expression was scored as positive when >10% of the cancer cells in tumor tissues showed cytoplasmic or nuclear staining.[19]

2.5 | Evaluation of the TSR

The TSR was visually determined on slides of pretreatment biopsies. TSR assessment was performed by two experienced pathologists. Only areas where both stromal and tumor cells were present on all

four sides of the microscopic field were scored, and the TSR was evaluated per 10-fold percentage (10%, 20%, etc.).

As in previous research,[18] a stroma percentage of slides less than or equal to 50% was assigned to the group with a low amount of stroma (i.e., TSR-high), and a stroma percentage of >50% was assigned to the group with a high amount of stroma (i.e., TSR-low).

2.6 | Statistical analysis

Statistical analysis was performed using SPSS (IBM). MCD Viewer (Fluidigm) was used to visualize the IMC data. Spearman rank-order correlation coefficients were calculated to assess associations. The chi-squared test was carried out to compare the differences between groups. Logistic regression analysis was applied to obtain the ORs and 95% confidence intervals (CIs) for pCR in accordance with TSR, vimentin expression, and their combination. A p-value <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

In total, 159 LARC patients (six assigned to the discovery group and 153 assigned to the validation group) who were treated with nCRT were included. Among the six patients in the discovery group, three achieved pCR, and among the 153 patients in the validation group, 32 achieved pCR. The detailed characteristics of the two groups of participants are shown in Table 1.

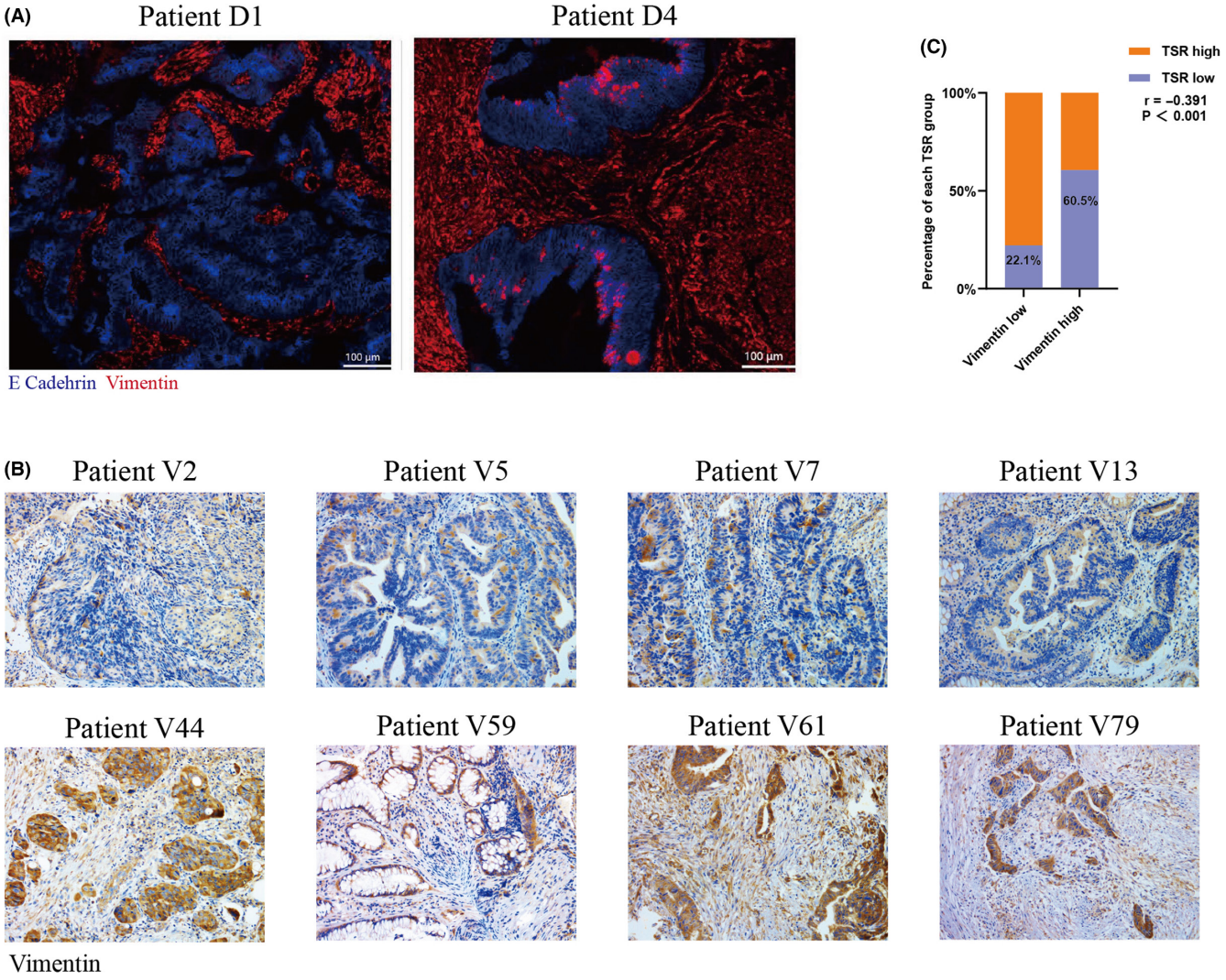

FIGURE 1 The association between vimentin and tumor–stroma ratio (TSR). (A) Representative mass cytometry images of LARC tissues from the discovery group (three patients from pCR group named patient D1–D3 and another three from the non-pCR group named patient D4–D6). (B) Representative immunohistochemical vimentin staining images of LARC tissues from the validation group (×200) (32 patients from the pCR group named patient V1–V32 and another 121 patients from the non-pCR group named patient V33–V153). (C) The distribution of low/high tumor–stroma ratio (TSR) in different vimentin expression

TABLE 2 The association between vimentin/TSR and clinicopathological features

	Vimentin			TSR		
Variables	Low	High	*p*	Low	High	*p*
Age, *N* (%)						
<55	47 (61.0)	35 (46.1)	0.063	34 (54.0)	48 (53.3)	0.938
≥55	30 (39.0)	41 (53.9)		29 (46.0)	42 (46.7)	
Gender, *N* (%)						
Male	56 (72.7)	52 (68.4)	0.559	45 (71.4)	63 (70.0)	0.849
Female	21 (27.3)	24 (31.6)		18 (28.6)	27 (30.0)	
BMI (kg/m^2), *N* (%)						
<25	63 (81.8)	60 (78.9)	0.655	49 (77.8)	74 (82.2)	0.496
≥25	14 (18.2)	16 (21.1)		14 (22.2)	16 (17.8)	
cT stage, *N* (%)						
cT3	56 (72.7)	49 (64.5)	0.271	37 (58.7)	68 (75.6)	0.027
cT4	21 (27.3)	27 (35.5)		26 (41.3)	22 (24.4)	
cN stage, *N* (%)						
cN0	35 (45.5)	22 (28.9)	0.035	15 (23.8)	42 (46.7)	0.004
cN+	42 (54.5)	54 (71.1)		48 (76.2)	48 (53.3)	
Tumor length (cm), *N* (%)						
<4	45 (58.4)	28 (36.8)	0.007	26 (41.3)	47 (52.2)	0.182
≥4	32 (41.6)	48 (63.2)		37 (58.7)	43 (47.8)	
DTAV (cm), *N* (%)						
<5	30 (39.0)	24 (31.6)	0.339	21 (33.3)	33 (36.7)	0.671
≥5	47 (61.0)	52 (68.4)		42 (66.7)	57 (63.3)	
Differentiation, *N* (%)						
Poor/Moderate	39 (50.6)	54 (71.1)	0.01	42 (66.7)	51 (56.7)	0.212
Well	38 (49.4)	22 (28.9)		21 (33.3)	39 (43.3)	
Histological type, *N* (%)						
Ulcerative	39 (50.6)	42 (55.3)	0.568	33 (52.4)	48 (53.3)	0.908
Other	38 (49.4)	34 (44.7)		30 (47.6)	42 (46.7)	
CEA (ng/ml), *N* (%)						
<5	55 (71.4)	53 (69.7)	0.818	42 (66.7)	66 (73.3)	0.373
≥5	22 (28.6)	23 (30.3)		21 (33.3)	24 (26.7)	
CA 19–9 (ng/ml), *N* (%)						
<39	71 (92.2)	74 (97.4)	0.284	60 (95.2)	85 (94.4)	0.828
≥39	6 (7.8)	2 (2.6)		3 (4.8)	5 (5.6)	
HB (g/L), *N* (%)						
<90	12 (15.6)	8 (10.5)	0.650	11 (17.5)	9 (10.0)	0.369
90–120 (male)/110 (female)	41 (53.2)	43 (55.6)		34 (54.0)	50 (55.6)	
≥120 (male)/110 (female)	24 (31.2)	25 (32.9)		18 (28.5)	31 (34.4)	

Abbreviations: BMI, body mass index; DTAV, distance from anal verge; CA 19–9, carbohydrate antigen 19–9; CEA, carcinoembryonic antigen; cN stage, clinical N stage; cT stage, clinical T stage; HB, hemoglobin; TSR, tumor–stroma ratio.

3.2 | Association between vimentin and TSR in tumors

To evaluate the relationship between vimentin and TSR, six patients whose tumor tissues were subjected to IMC in our previous study were assigned to the discovery group.[20] As shown in Figure 1A, we found a negative relationship between vimentin and TSR.

This relationship was further validated by IHC in a larger cohort of LARC patients (Figure 1B). A significantly negative correlation between vimentin and TSR was found ($r = -0.391$, $p < 0.001$). Among

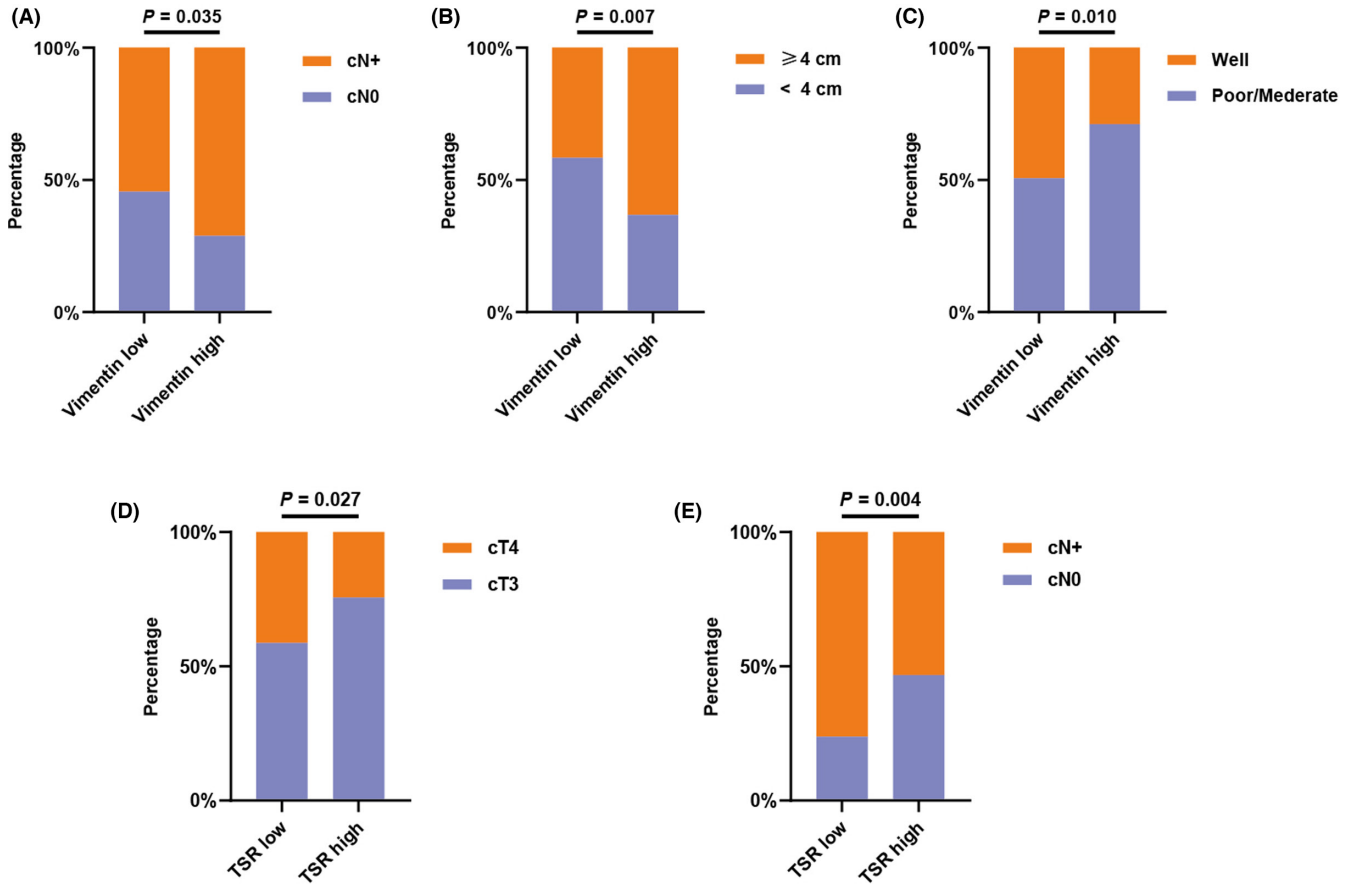

FIGURE 2 The distribution of (A) cN stage; (B) tumor length; and (C) differentiation of tumor in low/high vimentin groups. The distribution of (D) clinical T stage (cT) stage; and (E) clinical N stage (cN) stage in low/high tumor–stroma ratio (TSR) groups

the 76 vimentin-high patients, 46 (60.5%) had TSR-low tumors, and 60 (77.9%) of the 77 vimentin-low patients had TSR-high (Figure 1C).

3.3 | Associations between vimentin and TSR and clinicopathological features

The associations between vimentin and TSR and the clinicopathological features of LARC patients are shown in Table 2. Our results showed a strongly positive association between vimentin and nodal status ($p = 0.035$) (Figure 2A), as well as tumor length ($p = 0.007$) (Figure 2B). In addition, higher expression of vimentin was correlated with poorly differentiated tumors ($p = 0.010$) (Figure 2C). Concerning the TSR, significantly negative associations between cT stage ($p = 0.027$) (Figure 2D) and cN stage ($p = 0.004$) (Figure 2E), and TSR were also found in the current study.

3.4 | Predictive value of vimentin, TSR, and their combination for therapeutic response

In the discovery group (Figure 1A), we discovered a trend of lower vimentin and higher TSR in LARC patients who achieved pCR, while those who responded poorly to nCRT had vimentin-high/TSR-low tumors. These findings were further validated in 153 patients. In univariate analysis, patients with vimentin-low tumors had a 3.8 higher odds (1/0.260) of pCR after nCRT than patients with vimentin-high tumors (OR 0.260, 95% CI 0.102–0.602, $p = 0.002$), whereas patients with TSR-low tumors were significantly less likely to achieve pCR (OR 4.971, 95% CI 1.933–15.431, $p = 0.002$). In addition, our results showed that patients with higher BMI (OR 0.221, 95% CI 0.034–0.798, $p = 0.048$), longer tumor lengths (OR 0.395, 95% CI 0.170–0.875, $p = 0.025$), higher CEA levels (OR 0.279, 95% CI 0.079–0.770, $p = 0.024$), and accompanying nodal metastasis (OR 0.436, 95% CI 0.195–0.960, $p = 0.040$) had a significantly lower chance of achieving pCR. Whereas higher HB levels (90–120 (male)/110 (female): OR 4.470, 95% CI 0.827–83.229, $p = 0.159$; ≥120 (male)/110 (female): OR 8.382, 95% CI 1.512–157.416, $p = 0.047$) and more neoadjuvant chemotherapy cycles (3–4: OR 8.565, 95% CI 1.693–156.328, $p = 0.039$; 5–6: OR 7.800, 95% CI 0.883–168.444, $p = 0.090$) were found to be associated with a significantly higher odds of pCR (Table 3).

Multivariate analysis was corrected for significant factors (BMI, cN stage, tumor length, CEA, HB, and neoadjuvant chemotherapy cycles) in univariate analysis. Although both vimentin and TSR were no longer significant, there was still a clear trend with a 2.6 (1/0.377) lower odds of pCR if patients had vimentin-high tumors (OR 0.377, 95% CI 0.130–1.027, $p = 0.062$) and a 2.9 higher odds if patients

TABLE 3 Univariate logistic regression analysis of vimentin, TSR and the combination with respect to laser-assisted cartilage reshaping (LACR) patients' clinical responses to nCRT

Variables	*N*	OR	95% CI	*p*
Age				
<55	82	1.000		
≥55	71	0.873	0.393–1.908	0.735
Gender				
Male	108	1.000		
Female	45	0.924	0.374–2.138	0.857
BMI (kg/m^2)				
<25	123	1.000		
≥25	30	0.221	0.034–0.798	0.048
cT stage				
cT3	105	1.000		
cT4	48	0.993	0.414–2.257	0.987
cN stage				
cN0	57	1.000		
cN+	96	0.436	0.195–0.960	0.040
Tumor length (cm)				
<4	73	1.000		
≥4	80	0.395	0.170–0.875	0.025
DTAV (cm)				
<5	54	1.000		
≥5	99	1.840	0.790–4.685	0.175
Differentiation				
Poor/Moderate	93	1.000		
Well	60	1.490	0.674–3.278	0.320
Histological type				
Ulcerative	81	1.000		
Other	72	0.991	0.450–2.165	0.981
CEA (ng/ml)				
<5	108	1.000		
≥5	45	0.279	0.079–0.770	0.024
CA 19–9 (ng/ml)				
<39	145	1.000		
≥39	8	0.525	0.027–3.111	0.554
HB (g/L)				
<90	19			
90–120 (male)/110 (female)	84	4.470	0.827–83.229	0.159
≥120 (male)/110 (female)	50	8.382	1.512–157.416	0.047
Neo-chemo regime				
Capecitabine based	72	1.000		
5-Fluorouracil based	81	1.640	0.745–3.732	0.226
Neo-chemo cycles				
1–2	27	1.000		
3–4	112	8.565	1.693–156.328	0.039
5–6	14	7.800	0.883–168.444	0.090
Neo-radio technique				
IMRT	136	1.000		
3D-CRT	17	0.471	0.072–1.796	0.335
Neo-radio dose (Gy)				
<50	28	1.000		
≥50	125	0.963	0.371–2.830	0.941
Vimentin				
Low	77	1.000		
High	76	0.260	0.102–0.602	0.002
TSR				
Low	63	1.000		
High	90	4.971	1.933–15.431	0.002
Combination				
1	46	1.000		
2	47	5.211	1.248–35.582	0.042
3	60	11.846	3.197–77.079	0.001

Note: Combination: the combination of vimentin and TSR; 1: vimentin-high/TSR-low; 2: vimentin-low/TSR-low or vimentin-high/TSR-high; 3: vimentin-low/TSR-high.

Abbreviations: 3D-CRT, three-dimensional conformal radiotherapy; BMI, body mass index; CA 19–9, carbohydrate antigen 19–9; CEA, carcinoembryonic antigen; cN stage, clinical N stage; cT stage, clinical T stage; DTAV, distance from anal verge; HB, hemoglobin; IMRT, intensity-modulated radiation therapy; TSR, tumor–stroma ratio.

had TSR-high tumors (OR 2.910, 95% CI 0.976–9.995, $p = 0.067$). Moreover, no significant associations were found in other variables incorporated in multivariate analysis (Figure 3A) (Table 4).

Vimentin and TSR were combined to evaluate the possibility of an additional predictive effect. Three different combinations of vimentin and TSR (vimentin-high/TSR-low, vimentin-low/TSR-low or vimentin-high/TSR-high, and vimentin-low/TSR-high) were applied for the analysis. Patients with vimentin-low/TSR-low or vimentin-high/TSR-high (OR 5.211, 95% CI 1.248–35.582, $p = 0.042$) and vimentin-low/TSR-high (OR 11.846, 95% CI 3.197–77.079, $p = 0.001$) tumors showed a significantly higher chance of pCR than patients with vimentin-high/TSR-low tumors. These analyses show the strong predictive effect of the combination of TSR and vimentin for treatment response. Multivariate analysis showed that the combination of vimentin and TSR is an independent predictive factor for response to nCRT (vimentin-low/TSR-high: OR 9.324, 95% CI 2.290–63.623, $p = 0.006$). However, no statistical significance was found for vimentin-low/TSR-low or vimentin-high/TSR-high by multivariate logistic regression analyses, but a tendency of a higher odds of pCR was found (OR 4.165, 95% CI 0.918–29.733, $p = 0.092$) (Figure 3B) (Tables 3 and 4).

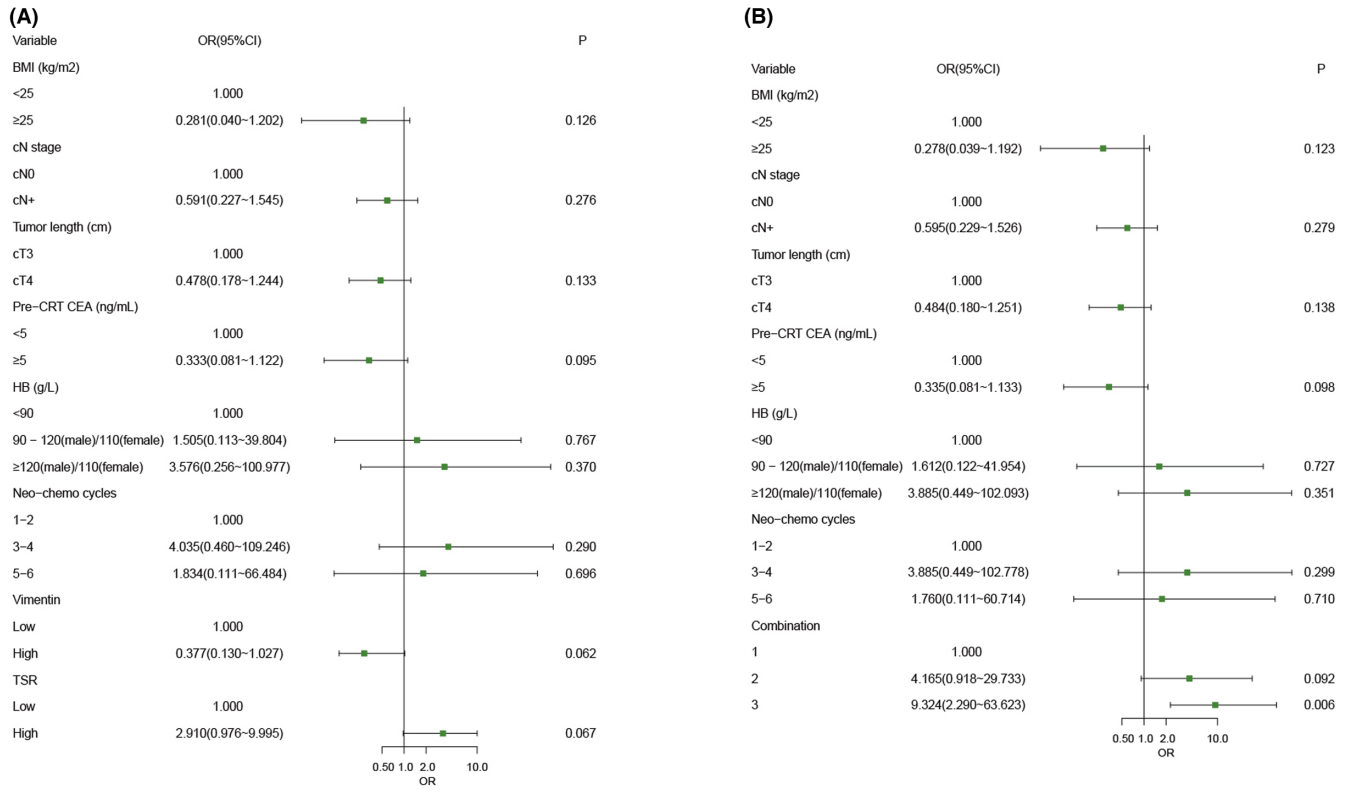

FIGURE 3 Forest plots for the potential predictive factors for treatment response in locally advanced rectal patients who received neoadjuvant chemoradiotherapy by (A) multivariate logistic analysis including vimentin and TSR; and (B) multivariate logistic analysis including the combination of vimentin and TSR. BMI, body mass index; CEA, carcinoembryonic antigen; cN stage, clinical N stage; HB, hemoglobin; TSR, tumor–stroma ratio. Combination: the combination of vimentin and TSR. 1: vimentin-high/TSR-low; 2: vimentin-low/TSR-low or vimentin-high/TSR-high; 3: vimentin-low/TSR-high

4 | DISCUSSION

As it was recommended as the standard treatment strategy, nCRT was demonstrated to significantly improve the prognosis of LARC patients.[1] However, therapy resistance occurred in the majority of LARC patients, with only 20% achieving pCR after receiving nCRT, similar to the results of the present study.[2,3] The risk factors are still poorly characterized despite the efforts of multiple studies. Further research is therefore needed to refine the nCRT outcome of LARC patients, to omit excessive treatment in some cases and to possibly escalate treatment for others.

To the best of our knowledge, this is the first study incorporating vimentin and TSR, markers from both tumor cells and the TME, to predict nCRT outcome in patients with LARC. We found that incorporating the expression of vimentin and TSR provided a superior prediction of treatment response compared with vimentin or TSR alone. When vimentin or TSR is solely assessed, neither vimentin nor TSR can independently predict the response to nCRT. When vimentin is combined with TSR, a group of patients with better therapeutic outcomes can be identified, namely, vimentin-low/TSR-high patients. Considering the possibility that this patient group may have a higher chemotherapy and radiotherapy sensitivity, the combined assessment of vimentin and TSR seems capable of identifying a group of patients who may benefit from more aggressive treatment.

EMT is well known to induce the progression of several types of cancers.[4–6] As a canonical biomarker of EMT and found to be associated with the aggressiveness of cancers, vimentin expressed on tumor tissues of LARC patients may predict the therapeutic outcome of nCRT.[7] Similar to our speculation, LARC patients with vimentin-high tumors had a greater chance of nonresponse. However, the expression of vimentin in LARC tissues in our study was unable to predict the treatment response independently, which means that other potential risk factors may be involved in the pathological process of vimentin that induces treatment resistance, and these findings may explain the paradoxical results about the role of vimentin in treatment response found by different studies.[10,11] Therefore, experimental studies are warranted to explore the mechanism by which vimentin mediates therapy resistance.

As discussed above, in the current study, we found a superior prediction of treatment response incorporating the expression of vimentin and TSR. Accumulating evidence has shown the important role of the TME in tumorigenesis, metastasis, and treatment resistance.[14,21,22] Therefore, predictive models for therapy response constructed based on biomarkers of tumor cells alone are

TABLE 4 Multivariate logistic regression analysis of vimentin, TSR and the combination for pCR in 153 LACR patients

		Vimentin/TSR			Combination		
Variables	***N***	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***
BMI (kg/m^2)							
<25	123	1.000			1.000		
≥25	30	0.281	0.040–1.202	0.126	0.278	0.039–1.192	0.123
cN stage							
cN0	57	1.000					
cN+	96	0.591	0.227–1.545	0.276	0.595	0.229–1.526	0.279
Tumor length (cm)							
<4	73	1.000					
≥4	80	0.478	0.178–1.244	0.133	0.484	0.180–1.251	0.138
CEA (ng/ml)							
<5	108	1.000					
≥5	45	0.000	0.081–1.122	0.095	0.335	0.081–1.133	0.000
HB (g/L)							
<90	19						
90–120 (male)/110 (female)	84	1.505	0.113–39.804	0.767	1.612	0.122–41.954	0.727
≥120 (male)/110 (female)	50	3.576	0.256–100.977	0.37	3.885	0.449–102.093	0.351
Neo-chemo cycles							
1–2	27	1.000			1.000		
3–4	112	4.035	0.460–109.246	0.29	3.885	0.449–102.778	0.299
5–6	14	1.834	0.111–66.484	0.696	1.760	0.111–60.714	0.71
Vimentin							
Low	77	1.000					
High	76	0.377	0.130–1.027	0.062			
TSR							
Low	63	1.000					
High	90	2.910	0.976–9.995	0.067			
Combination							
1	46				1.000		
2	47				4.165	0.918–29.733	0.092
3	60				9.324	2.290–63.623	0.006

Note: Combination: the combination of vimentin and TSR. 1: vimentin-high/TSR-low; 2: vimentin-low/TSR-low or vimentin-high/TSR-high; 3: vimentin-low/TSR-high.

Abbreviations: BMI, body mass index; CEA, carcinoembryonic antigen; cN stage, clinical N stage; HB, hemoglobin; TSR, tumor–stroma ratio.

insufficient, and incorporating biomarkers from both tumor cells and the TME may provide additive significance. The synergetic role of TSR and vimentin was shown by our results in predicting the response to nCRT of LARC patients. In addition, incorporating TSR with vimentin in clinical practice has certain advantages because it can be carried out on the same IHC slide of vimentin staining during assessment.

Therefore, the addition of TSR/vimentin in clinical practice may be an easy but powerful way to help with the decision of whether to prescribe neoadjuvant treatment to LARC patients. For patients with TSR-low/vimentin-high tumors, it might be worthwhile to consider alternative or additional treatments.

In the current study, we observed higher vimentin expression in tumor tissues with a high stromal content than in those with a low stromal content. These associations can be explained by the cross-talk between tumor cells and the TME. Fibroblasts, which are the main cellular component within the tumor–stroma, express higher levels of all cancer-associated fibroblast (CAF) markers in stroma-high tumors.[23] Through the release of secreted paracrine factors and remodeling of the extracellular matrix (ECM), CAFs play a role in tumor promotion, including the requirement of the EMT phenotype in tumor cells.[24–26] In addition, tumor-infiltrating immune cells, such as tumor-associated macrophages and myeloid-derived suppressor cells, are also involved in the process of EMT.[27,28] Regarding

clinicopathological features, vimentin expression was higher in tumors that were poorly differentiated and was positively correlated with nodal stage and tumor length, whereas TSR-low tumors were associated with more advanced T stage and lymph node metastasis. These results imply their potential involvement in tumor differentiation, invasion, and metastasis, which also suggests that vimentin expression and TSR might serve as early biomarkers for disease progression in patients with LARC.

This study had several limitations. First, this was a retrospective study. Second, a relatively small cohort of patients was included in our current study; therefore, some potential risk factors, such as T stage and distance from anal verge, failed to show statistical significance. Therefore, these results must be confirmed in larger cohorts and through multicenter studies. In addition, only correlation analyses were performed in our study, and the potential mechanism of these correlations should be further explored by experimental studies.

In conclusion, in LARC patients treated with nCRT, those with vimentin-low/TSR-high in pretreatment biopsy samples of the tumor were associated with a higher pCR rate, and the combination of vimentin and TSR in pretreatment tumor tissue can provide superior value to predict LARC patients who are suitable for nCRT. We believe that our study will provide a foundation for developing new predictive biomarkers and might aid in clinical decision-making regarding the delivery of improved therapies for LARC.

AUTHOR CONTRIBUTIONS

Hong Zhao, Qingling Zhang, and Ying Huang conceived and designed the experiments. Wenjing Tian, Yuqin Yang, Qi Qin, Liguo Zhang, Zheyan Wang, Liqian Su, Lirong Zeng, Hui Chen, Lingzhi Hu, Jiawei Hong conducted, and analyzed the data. Wenjing Tian, Yuqin Yang, and Qi Qin wrote this manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The data are not publicly available due to privacy or ethical restrictions and available on reasonable request from the corresponding author.

DISCLOSURE

None of the authors of this manuscript is a current Editor or Editorial Board Member of Cancer Science.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: The studies conform to the provisions of the Declaration of Helsinki, and were reviewed and approved by the Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University and Harbin Medical University Cancer Hospital. Informed Consent: Written informed consent was gained from all participants.

ORCID

Yuqin Yang https://orcid.org/0000-0002-4458-3979
Qi Qin https://orcid.org/0000-0002-4934-5136

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