Clinicopathological significance and prognostic analysis of p21 and EGFR in colorectal cancer: a retrospective analysis on 12 319 cases in China

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ABSTRACT

Aims Colorectal cancer (CRC) is the third most common malignancy worldwide. Accurate pathological diagnosis and predictive abilities for treatment response and prognosis are crucial for patients with CRC. This study aims to analyse the expressions of p21 and EGFR in CRC and their relationships with clinicopathological characteristics and prognosis to enhance diagnostic and prognostic evaluations.

Methods This study conducted a retrospective analysis of p21 and EGFR expressions in 12 319 Chinese patients with CRC using immunohistochemistry. The relationships between these expressions and clinicopathological characteristics and survival outcomes were explored through statistical and survival analyses.

Results Differential expressions of p21 and EGFR in CRC were closely related to clinicopathological characteristics and significantly impacted overall survival (OS). p21 expression was associated with the primary tumour site, mucinous subtype, lymphovascular invasion, perineural invasion, circumferential resection margin, T stage, N stage, tumour, node, metastases (TNM) stage, and mismatch repair status. EGFR expression was related to mucinous subtype, tumour differentiation, lymphovascular invasion, perineural invasion, tumour size, T stage, N stage, TNM stage and *BRAF* gene mutation. p21 and EGFR expressions were positively correlated (r=0.11). High p21 expression correlated with favourable OS, whereas high EGFR expression predicted poorer OS. A prognostic nomogram incorporating these biomarkers and clinical variables demonstrated robust predictive power for patient survival rates.

Conclusion p21 and EGFR serve as potential indicators for pathological diagnosis, risk stratification, and predicting treatment efficacy and prognosis in patients with CRC. The study's findings provide valuable references for personalised treatment and prognosis evaluation in clinical practice.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Colorectal cancer (CRC) is a prevalent malignancy globally, necessitating accurate pathological diagnosis and prognosis prediction. p21 regulates the cell cycle and immunosurveillance, while EGFR plays a crucial role in cell proliferation, invasion and angiogenesis, with its overexpression linked to tumour growth and poor prognosis. The specific implications of these biomarkers in CRC, particularly regarding clinicopathological characteristics and prognosis in large patient cohorts, required further investigation.

WHAT THIS STUDY ADDS

⇒ This study, involving 12 319 Chinese patients with CRC, reveals that differential expressions of p21 and EGFR are significantly associated with clinicopathological features and overall survival. A positive correlation between p21 and EGFR was also identified. The study demonstrates that a prognostic nomogram incorporating these biomarkers and clinical variables has strong predictive power for patient survival rates.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings highlight the potential of p21 and EGFR as biomarkers for CRC diagnosis, risk stratification and prognosis. These biomarkers can guide personalised treatment strategies, improving patient outcomes, and emphasise the importance of integrating molecular and clinicopathological data in CRC management.

INTRODUCTION

Colorectal cancer (CRC) is the third leading malignancy on a global scale,with than 1.9 million new cases annually and an alarming increase in both incidence and mortality.[1] CRC commonly occurs in middle-aged people, with about 60–65% of cases being sporadic and 35–40% attributed to genetic factors.[2] Surgical treatment is the primary option for early-stage CRC, and the advent of molecularly targeted and immunotherapeutic agents has significantly enhanced clinical outcomes.[3] However, early symptoms of CRC are often subtle, and the disease is usually advanced by the time symptoms like bloody stools and abdominal pain appear, with no standard methods to predict treatment efficacy and prognosis.[4] Therefore, providing precise pathological diagnosis and improving predictive abilities for treatment response and prognosis in patients with CRC are crucial.

The academic inquiry of cancer development has entered the stages of molecular biology and genetics. p21 and EGFR, key molecules in cell regulation, have received significant scholarly focus due to their implications in tumour genesis and progression. p21 is a cyclin-dependent kinase inhibitor with immunosurveillance functions in senescent cells.[5] EGFR, a transmembrane tyrosine kinase receptor, plays a crucial role in signalling pathways and is involved in cell proliferation, invasion and angiogenesis.[6]

This study focuses on the clinicopathological significance and prognostic analysis of p21 and EGFR in CRC, aiming to provide references for pathological diagnosis and prognosis in patients with CRC.

MATERIALS AND METHODS

Patient selection

This study conducted a retrospective analysis of pathological diagnostic reports and immunohistochemistry reports from 12319 patients with confirmed postoperative pathology of CRC at the Fudan University Shanghai Cancer Center in China between January 2008 and December 2020. These data were retrieved from the hospital's case archive and formatted for analysis. Inclusion criteria: (1) patients aged 18 years and above; and (2) patients with CRC as the primary site of the tumour, including those with metastases. Exclusion criteria: (1) patients with primary tumours originating from other organs that have metastasised to the colon; and (2) patients with autoimmune diseases. Tumour, node, metastases (TNM) staging was performed using the eighth edition of the American Joint Committee on Cancer staging standards.

Patients were followed up post-surgery through regular clinic visits or phone calls until September 2022, with a follow-up duration of 0.6–281 months and a median follow-up time of 47.6 months. Overall survival (OS) was used as a prognostic indicator.

Pathological evaluation

Pathological slides were reviewed by two physicians. Immunohistochemistry was scored based on the intensity of staining and the percentage of positive cells, classified as negative, weakly positive and positive. Clear absence of discernible staining is indicative of negativity. Faint, focal light brown staining observed in some cells represents weak positivity, while widespread, brown-yellow staining seen throughout the cells signifies positivity. Samples that failed the analysis due to insufficient or low-quality material were excluded.

Statistical analysis

Data were analysed using SPSS (V.25.0). Differences in counts data between groups were compared using the $\chi2$ test. Correlation analysis for ordered categorical variables was conducted using Spearman's correlation analysis. Kaplan-Meier survival curves and log-rank tests were used for prognostic analysis. On the basis of univariate and multivariate Cox regression analyses, a nomogram prediction model was constructed using R (V.4.2.1). All statistical tests were two sided, and $p<0.05$ was considered statistically significant.

RESULTS

Expression levels and distribution of p21 and EGFR in patients with CRC

Staining intensity varies in correlation with the expression levels of tumour cells. Based on the depth of the immunostaining colour and the proportion of positive cells, the expression levels of p21 and EGFR were categorised as negative, weakly positive and positive. Representative histological images and the distribution of p21 and EGFR expression are shown in figure 1. p21 staining is primarily located in the nuclei, whereas EGFR staining mainly occurs on the cell membrane. In the negative group, no discernible staining is observed; in the weakly positive group, some cells exhibit faint, focal light brown staining; and in the positive group, widespread brown-yellow staining is generally visible. In our study, p21 expression was negative in 33% of

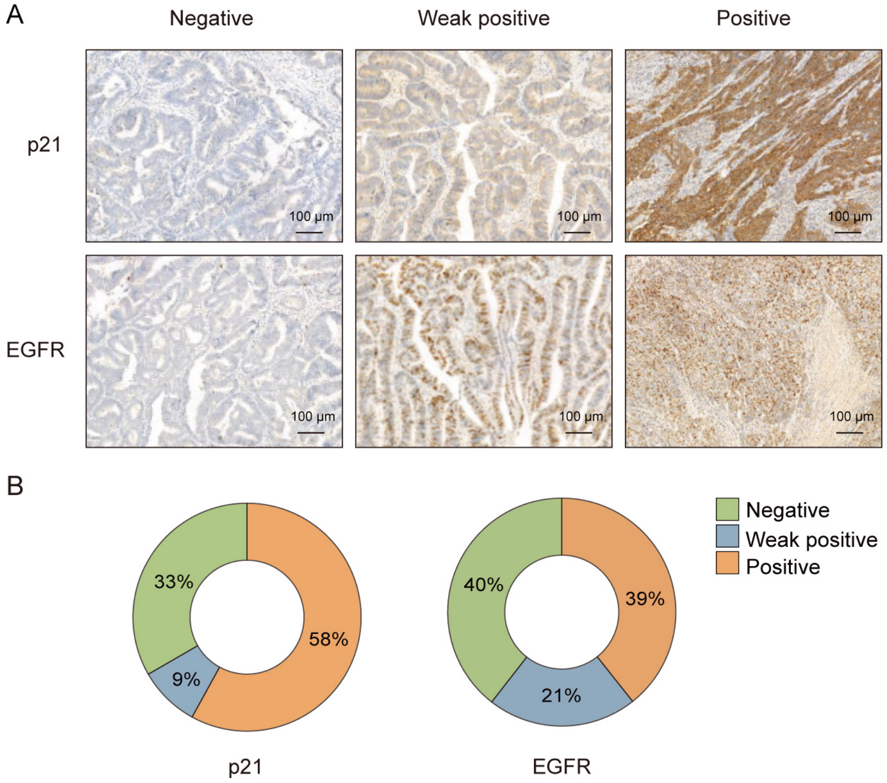

Figure 1 Representative histological images (A) and distribution analysis (B) of p21 and EGFR in patients with colorectal cancer.

CRC samples, weakly positive in 9% of the samples and positive in 58% of the samples. For EGFR protein, the proportions were 40% negative, 21% weakly positive and 39% positive.

Correlation of p21 and EGFR expressions with clinicopathological characteristics

This study collected 12 319 colorectal cancer specimens, including 4926 males and 7393 females, aged 18–95 years, with an average age of 59.46±12.08 years. The results showed that the differential expressions of p21 and EGFR are closely related to the clinicopathological characteristics of CRC (table 1).

The differential expression of p21 was associated with primary tumour site, mucinous subtype, lymphovascular invasion, perineural invasion, circumferential resection margin, T stage, N stage, TNM stage and mismatch repair of patients with CRC ($p<0.05$), but not with age, gender, tumour differentiation, tumour size, M stage, microsatellite instability, *KRAS* gene, *NRAS* gene and *BRAF* gene mutation ($p>0.05$).

The differential expression of EGFR was related to mucinous subtype, tumour differentiation, lymphovascular invasion, perineural invasion, tumour size, T stage, N stage, TNM stage and *BRAF* gene mutation ($p<0.05$), but not with age, gender, primary tumour site, circumferential resection margin, M stage, mismatch repair, microsatellite instability, *KRAS* gene and *NRAS* gene mutation ($p>0.05$).

Expression concordance between p21 and EGFR in CRC

In this group, 916 patients showed co-negativity, and 2105 patients co-positivity for p21 and EGFR. Spearman's correlation analysis revealed a positive correlation between p21 and EGFR expression ($r=0.11$) with statistical significance ($p<0.001$) (table 2).

Survival outcomes associated with p21 and EGFR expressions

Postoperative follow-up classified patients into low expression (negative expression) and high expression (weakly positive and positive) groups for survival analysis. Results indicated that p21 and EGFR expression differences significantly impacted patient OS in CRC. Kaplan-Meier analysis showed that high p21 expression was associated with significantly higher OS compared with low expression ($p=0.004$), whereas high EGFR expression was associated with lower OS ($p=0.009$) (figure 2), suggesting p21 and EGFR as potential prognostic indicators.

Cox regression analysis of prognostic factors in CRC

Univariate Cox proportional hazards regression model identified significant factors affecting prognosis of patients with CRC, including age, mucinous subtype, tumour differentiation, lymphovascular invasion, perineural invasion, circumferential resection margin, tumour size, T stage, N stage, M stage, TNM stage, p21 expression and EGFR expression ($p<0.05$). Multivariate analysis revealed independent risk factors: age ≥50 years, poor tumour differentiation, lymphovascular invasion, perineural invasion, positive circumferential resection margin, tumour size ≥4 cm, advanced N stage, advanced M stage, advanced TNM stage and low p21 expression ($p<0.05$) (table 3), whereas high EGFR expression was not an independent prognostic factor.

Nomogram model for predicting survival of patients with CRC

Based on Cox regression analysis, a nomogram prediction model for survival rates of patients with CRC was constructed, integrating various prognostic factors. The total score obtained by

Table 1 Relationship between p21, EGFR expression and clinicopathological characteristics of colorectal cancer

Clinicopathological characteristics	p21 expression				EGFR expression			
	–	+	++	P value	–	+	++	P value
Age (years)				0.666				0.502
<50	830	203	1441		578	294	543	
≥50	3274	863	5698		2490	1354	2514	
Gender				0.448				0.184
Female	1647	407	2869		1224	617	1222	
Male	2457	659	4270		1844	1031	1835	
Primary tumour site				<0.001*				0.479
Rectum	2250	499	3792		1640	852	1585	
Distal	904	200	1665		695	390	685	
Proximal	929	192	1669		677	376	740	
Synchronous	12	175	0		52	27	44	
Mucinous subtype				<0.001*				<0.001*
Negative	2777	745	5501		2203	1078	1856	
Positive	390	79	569		236	101	83	
Tumour differentiation				0.107				0.001*
Poor	50	14	101		44	21	33	
Moderate	2877	751	5133		2186	1176	2075	
Well	1043	288	1692		750	418	889	
Lymphovascular invasion				0.025*				<0.001*
Negative	2853	757	5149		2277	1170	2103	
Positive	1192	306	1915		785	477	947	
Perineural invasion				<0.001*				<0.001*
Negative	2920	755	5373		2314	1185	2095	
Positive	1130	311	1707		751	461	956	
Circumferential resection margin				0.045*				0.053
Negative	3615	1020	6656		2971	1588	2876	
Positive	85	17	110		35	20	55	
Tumour size (cm)				0.215				0.005*
<4	2879	733	4900		2165	1167	2053	
≥4	1206	330	2209		892	479	995	
T stage				<0.001*				<0.001*
Tis	16	5	26		14	8	10	
T1	152	48	296		150	71	102	
T2	677	181	1204		530	266	463	
T3	940	499	2649		1116	903	2031	
T4	2307	331	2946		1251	399	440	
N stage				0.002*				0.014*
N0	1929	545	3634		1623	825	1491	
N1	1347	332	2201		937	539	986	
N2	826	189	1303		508	284	579	
M stage				0.522				0.441
M0	3550	933	6222		2661	1408	2629	
M1	554	133	917		407	240	428	
TNM stage				0.021*				0.045*
0	13	4	21		13	7	6	
I	595	166	1117		497	241	418	
II	1192	334	2277		1001	524	959	
III	1747	429	2796		1147	635	1241	
IV	554	133	917		407	240	428	
Mismatch repair				<0.001*				0.609
pMMR	3087	956	6159		2824	1522	2806	
dMMR	895	103	892		225	114	236	
Microsatellite instability				0.402				0.197
MSS	37	15	94		46	29	41	
MSI-H	5	1	8		2	6	3	
MSI-L	1	2	3		1	1	3	
KRAS				0.639				0.449
Wild type	443	194	627		345	267	499	
Mutant type	382	148	520		277	214	448	
NRAS				0.595				0.95

Continued

Table 1 Continued

Clinicopathological characteristics	p21 expression –	p21 expression +	p21 expression ++	P value	EGFR expression –	EGFR expression +	EGFR expression ++	P value
Wild type	684	326	997		549	469	911	
Mutant type	23	10	25		17	13	26	
BRAF				0.166				0.004*
Wild type	729	326	996		568	463	882	
Mutant type	23	12	50		10	17	47	

– represents negative, + represents weakly positive, ++ represents positive.
*P<0.05.
dMMR, deficient mismatch repair; MSI-H, high microsatellite instability; MSI-L, low microsatellite instability; MSS, microsatellite stability; pMMR, proficient mismatch repair; TNM, tumour, node, metastases.

Table 2 Spearman's correlation analysis of p21 and EGFR levels

EGFR	p21 Negative	p21 Weakly positive	p21 Positive	r	P value
Negative	916	411	1738	0.11	<0.001
Weakly positive	446	221	976		
Positive	650	300	2105		

adding individual scores predicts the 1-year, 3-year and 5-year survival rates for patients with CRC (figure 3). The model, combining different pathological parameters, offers better predictive performance and clinical applicability, translating regression equations into a visual format for patient assessment.

DISCUSSION

The incidence of CRC is influenced by diet, social environment, genetics and other factors. The disease is typically asymptomatic in its early stages, while later stages may present with bloody or pus-filled stools, diarrhoea, constipation and other adverse symptoms. Current treatments are limited for patients with large tumours, severe local infiltration or widespread metastasis.[7] Molecular testing plays a crucial role in tumour pathological diagnosis, risk stratification, treatment monitoring and prognosis prediction.

p21 and EGFR play important roles in the occurrence and development of cancer. Their expression and function have significant clinical relevance in the diagnosis, prognosis assessment and selection of treatment strategies for cancer. Currently, targeted therapies for EGFR mainly include tyrosine kinase inhibitors (TKIs) and monoclonal antibodies. EGFR-TKIs act primarily on the intracellular tyrosine kinase domain of the EGFR receptor, blocking its kinase activity and thereby inhibiting downstream signal transduction. EGFR-TKIs include drugs from several generations: the first generation includes gefitinib, erlotinib and icotinib; the second generation includes afatinib and dacomitinib; and the third generation includes osimertinib and amivantamab. EGFR monoclonal antibodies, by binding to the extracellular domain of the EGFR receptor and preventing its natural ligands from binding, can also effectively block the EGFR-mediated signalling pathway. Commonly used EGFR monoclonal antibodies include cetuximab, necitumumab, panitumumab and nimotuzumab. In contrast, targeted therapies for p21 remains limited. The known p21 inhibitor UC2288 is synthesised based on the chemical structure model of sorafenib. Targeting other genes in the p21 cascade to induce the expression of p21 is seen as a potential strategy to inhibit tumour growth and metastasis.

Protein p21, expressed by the *CDKN1A* gene, is a cyclin-dependent kinase inhibitor that can inhibit the activity of cyclin-dependent kinases and proliferating cell nuclear antigen.[8] The classic tumour suppressor protein P53 plays a key transcriptional regulatory role in the cell cycle checkpoint, apoptosis and senescence, promoting the expression of p21 by binding to two sites upstream of the p21 promoter. High levels of p21 result from P53 or mitogen stimulation, leading to the formation of the Rb–E2F protein complex and downregulation of numerous cell cycle-related proteins, causing cell cycle arrest in the G1 phase until damaged DNA is repaired.[9] p21 also acts as an immunosurveillance 'scout', promoting the clearance of senescent cells to ensure homeostasis.[5] Clinical studies have reported that high p21 expression is associated with better prognosis in cancers such as urothelial carcinoma, breast cancer, bladder cancer, oesophageal cancer and ovarian cancer.[10–14] Research in CRC mouse models found that p21 deficiency in Th1 cells promotes tumour growth, suggesting p21's vital role in regulating T cell effector functions and preventing DNA damage accumulation in highly proliferative effector CD4+ T cells, with low p21 expression in tumour-infiltrating CD4+ T cells correlated with shorter survival in patients with CRC.[15] Research found that

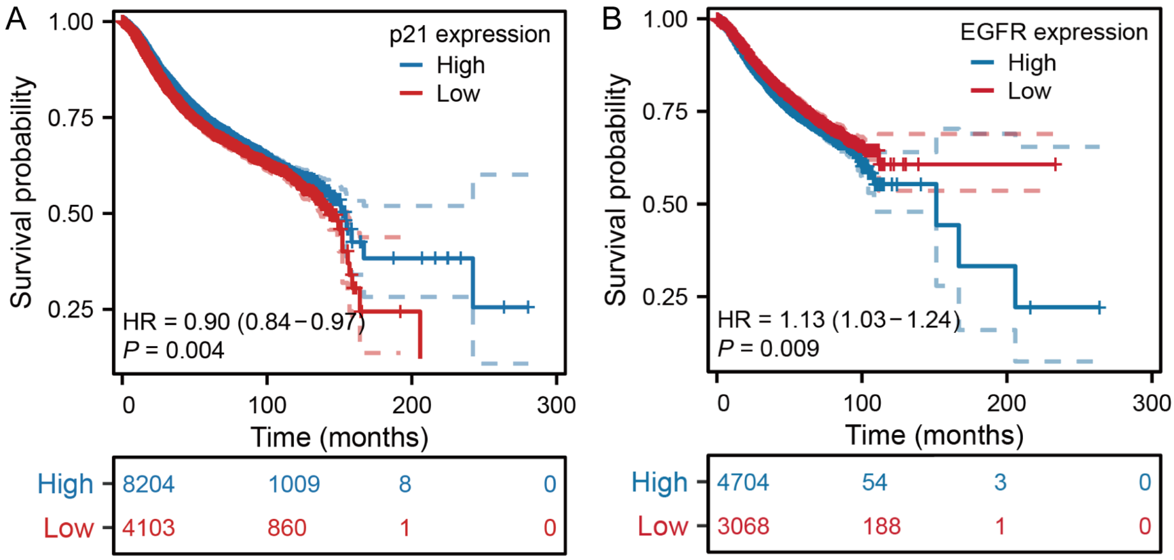

Figure 2 Correlation of p21 (A) and EGFR (B) expression levels with overall survival in patients with colorectal cancer.

Table 3 Univariate and multivariate Cox regression analyses for CRC prognostics

	Cox univariate analysis		Cox multivariate analysis	
Clinicopathological characteristics	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Age (≥50 years vs <50 years)	1.264 (1.162 to 1.375)	<0.001	1.394 (1.212 to 1.603)	<0.001
Gender (female vs male)	0.939 (0.880 to 1.001)	0.055	1.000 (0.899 to 1.113)	0.999
Mucinous subtype (positive vs negative)	1.271 (1.144 to 1.412)	<0.001	1.033 (0.853 to 1.253)	0.737
Tumour differentiation (poor vs moderately well)	1.972 (1.845 to 2.108)	<0.001	1.300 (1.154 to 1.465)	<0.001
Lymphovascular invasion (positive vs negative)	2.471 (2.318 to 2.633)	<0.001	1.398 (1.241 to 1.575)	<0.001
Perineural invasion (positive vs negative)	2.272 (2.129 to 2.424)	<0.001	1.573 (1.406 to 1.761)	<0.001
Circumferential resection margin (positive vs negative)	4.303 (3.695 to 5.012)	<0.001	2.407 (1.834 to 3.160)	<0.001
Tumour size (≥4 cm vs <4 cm)	1.231 (1.152 to 1.315)	<0.001	1.279 (1.143 to 1.432)	<0.001
T stage (T2, T3 and T4 vs Tis and T1)	3.086 (2.428 to 3.922)	<0.001	1.312 (0.859 to 2.005)	0.208
N stage (N1 and N2 vs N0)	3.150 (2.938 to 3.378)	<0.001	1.720 (1.504 to 1.967)	<0.001
M stage (M1 vs M0)	6.252 (5.839 to 6.694)	<0.001	4.470 (3.979 to 5.022)	<0.001
TNM stage (II, III and IV vs 0 and I)	3.620 (3.163 to 4.143)	<0.001	1.464 (1.124 to 1.908)	0.005
p21 (weakly positive and positive vs negative)	0.904 (0.844 to 0.968)	0.004	0.816 (0.722 to 0.923)	0.001
EGFR (weakly positive and positive vs negative)	1.128 (1.031 to 1.235)	0.009	1.002 (0.900 to 1.115)	0.977

RNA-binding protein PUMILIO promotes cancer cell growth by suppressing p21 expression in CRC.[16] Consistent with these findings, this study shows that patients with CRC with high p21 expression have significantly higher OS compared with those with low expression. The expression differences in p21 are associated with primary tumour site, mucinous subtype, lymphovascular invasion, perineural invasion, circumferential resection margin, T stage, N stage, TNM stage and mismatch repair. Cox multivariate regression analysis identifies low p21 expression as an independent risk factor affecting patient prognosis.

Current research widely suggests that overexpression and hyperactivation of EGFR are responsible for tumour cell growth, apoptosis resistance, angiogenesis and metastasis.[17] EGFR tyrosine kinase activity is influenced by various carcinogenic factors, including *EGFR* gene mutations, increased gene copy number and overexpression of the EGFR protein. When activated, EGFR triggers a cascade in downstream signalling pathways, including the MAPK, JAK/STAT and PI3K/Akt pathways.[18] Reports from European Society for Medical Oncology in 2023 summarised survival data from previous clinical studies using EGFR inhibitors for the treatment of patients with RAS/BRAF wild-type metastatic colorectal cancer, indicating that about one-third of patients significantly benefit from EGFR inhibitor treatment.[19–22] This study shows that patients with high EGFR expression have significantly lower OS compared with those with low expression, consistent with previous studies. The analysis indicates that EGFR expression differences correlate with mucinous subtype, tumour differentiation, lymphovascular invasion, perineural invasion, tumour size, T stage, N stage, TNM stage and *BRAF* gene mutation, with high EGFR expression identified as a risk factor affecting prognosis in univariate Cox regression analysis.

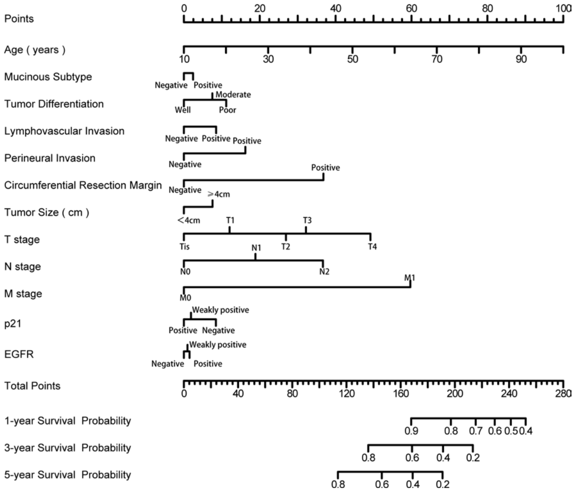

Figure 3 Nomogram model for the survival rates of patients with colorectal cancer.

In addition, research indicated that the expression of p21 in human bronchial epithelial cells depends on the activation of EGFR.[23] When EGFR inhibitors were used on the cells, p21 expression was significantly reduced. Through a statistical analysis of 426 patients with liver cancer, EGFR mutation was found to play a significant role in the regulation of p21, exhibiting a positive RNA level correlation between the two.[24] This is consistent with the findings of this study where a significant positive correlation exists between p21 and EGFR expression in patients with CRC. However, the specific mechanisms behind this correlation in CRC require further research.

This article discusses the clinicopathological significance of p21 and EGFR in CRC and their prognostic value, revealing that their differential expression is closely related to clinicopathological characteristics and is significantly correlated with OS, offering predictive value for patient treatment outcomes and prognosis. A nomogram prediction model incorporating various factors improves predictive performance and clinical utility. The elderly, mucinous subtype positive, tumour poorly differentiated, lymphovascular invasion positive, perineural invasion positive, circumferential resection margin positive, tumour size ≥4 cm, advanced TNM staging, low p21 expression and high EGFR expression in patients with CRC are associated with higher total scores in the nomogram prognostic model, resulting in lower survival rates.

In conclusion, p21 and EGFR are involved in the pathophysiological process of CRC development, providing indications for clinicopathological diagnosis and risk stratification, and may serve as potential indicators for predicting patient treatment efficacy and prognosis.

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Contributors YF designed the study, contributed to data analysis and interpretation, and approved the final version of the manuscript to be published.

MM participated in data collection and contributed to the initial drafting of the manuscript. LG was involved in data collection and analysis, and contributed to the preparation of materials. MX was responsible for the acquisition and analysis of data. YY and DH both provided critical revisions. WS is responsible for the content as guarantor. All authors have read and agreed to the published version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Ethics Committee of Fudan University Shanghai Cancer Center (IRB approval no. 050432-4-2108*).Written informed consent was obtained from the patients for publication and any accompanying images.

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