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Plasma Leucine-Rich Alpha-2-Glycoprotein 1 Reflects Higher Histological Grade, Worse Disease-Free Survival, and Unfavorable Overall Survival in Colorectal Cancer Patients who Receive Tumor Resection

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Leucine-rich alpha-2-glycoprotein 1 (LRG1) promotes colorectal cancer (CRC) growth, migration, and invasion. This study intended to investigate the association of plasma LRG1 with clinical characteristics, disease-free survival (DFS), and overall survival (OS) in CRC patients who received tumor resection. This study retrospectively included 125 CRC patients who received tumor resection. LGR1 level was detected in their preoperative plasma samples via enzyme-linked immunosorbent assay. The median level of plasma LRG1 was 53.4 μg/mL (quartile 1: 34.0 μg/mL, quartile 3: 102.5 μg/mL). Plasma LRG1 was elevated in patients with high histological grade versus those with low grade (P = 0.005). Plasma LRG1 was varied among patients with different node (P = 0.004) and tumor-node-metastasis (P = 0.001) stages. Moreover, plasma LRG1 ≥ 50 μg/mL (at around the median level) was not related to DFS (P = 0.074) or OS (P = 0.077). While plasma LRG1 ≥ 100 μg/mL (at around the quartile 3 level) was linked with shortened DFS (P = 0.018) and OS (P = 0.016). The 3-year accumulating DFS and OS rates were 60.8% and 64.4% in patients with plasma LRG1 ≥ 100 μg/mL; they were 75.7% and 82.9% in patients with plasma LRG1 < 100μg/mL, respectively. Furthermore, plasma LRG1 ≥ 100 μg/mL (hazard ratio (HR): 2.728, P = 0.036) and age ≥ 60 years (HR: 2.815, P = 0.041) were independently associated with shortened DFS. Only node stage (HR: 3.150, P = 0.004) was independently linked with shortened OS. In conclusion, LRG1 is associated with elevated histological grade and worse DFS and OS, with its level ≥ 100 μg/mL as an independent factor for shortened DFS in CRC patients who receive tumor resection.

Keywords: clinical characteristics; colorectal cancer with tumor resection; disease-free survival; leucine-rich alpha-2-glycoprotein 1; overall survival
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Introduction

Colorectal cancer (CRC) is the third most frequent malignancy and the second leading cause of cancer-related death worldwide, with approximately 1.9 million new cases and 0.9 million new deaths globally in 2020 (Sung et al. 2021). The factors associated with CRC occurrence include unhealthy lifestyles, obesity, older age, males, and others (Ionescu et al. 2023). Surgical resection is the standard approach for CRC patients without distant metastases (Benson et al. 2021, 2022). Worryingly, the long-term survival outcomes of CRC patients who receive tumor resection exhibit considerable heterogeneity (Jiang et al. 2021; Siegel et al. 2023). In detail, the 5-year disease-free survival (DFS) rate and overall survival (OS) rate of CRC patients who receive tumor resection range from 61.0% to 91.0% and from 47.7% to 91.1%, respectively (Kong et al. 2022; Warps et al. 2022; Chen et al. 2023; Ishiyama et al. 2023; Liu et al. 2023). Therefore, finding some biomarkers to predict survival in CRC patients who receive tumor

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resection is crucial for tailoring personalized management and improving their outcomes.

Leucine-rich alpha-2-glycoprotein 1 (LRG1), with length of 312 amino acids and molecular weight of 45 kD, is a regulator for transforming growth factor-β (TGF-β) signaling pathway (Zou et al. 2021; Camilli et al. 2022). It is reported that LRG1 participates in many processes of CRC, including angiogenesis, migration, and invasion (Zhang et al. 2016; Zhou et al. 2017; Zhang et al. 2018; Zhu et al. 2023). For example, LRG1 facilitates CRC angiogenesis by modulating TGF-β signaling and the subsequent phosphorylation of Smad1/5 (Zhu et al. 2023). Another study indicates that LRG1 promotes invasion and induces epithelial-mesenchymal transition (EMT) of CRC cells by upregulating hypoxia-inducible factor-1α (Zhang et al. 2016). In addition, silence of LRG1 inhibits growth and migratory ability of CRC cells (Zhang et al. 2018). Clinically, some studies show the prognostic ability of LRG1 in stage III CRC patients who receive tumor resection (Sun et al. 2017; Zhong et al. 2019). In one study, serum LRG1 is correlated with tumor recurrence in stage III colon cancer patients who receive tumor resection (Zhong et al. 2019). In another study, tumor LRG1 is associated with worse cancer differentiation as well as shortened DFS and OS in stage III CRC patients who receive tumor resection (Sun et al. 2017). However, these two studies only enroll patients at state III (Sun et al. 2017; Zhong et al. 2019); therefore, the capability of LRG1 in predicting the prognosis in the general population of CRC patients who receive tumor resection remains uncertain.

Hence, this study intended to investigate the association of plasma LRG1 with clinical characteristics, DFS, and OS in CRC patients who received tumor resection.

Methods

CRC patients

A sum of 125 patients with CRC who received tumor resection between August 2019 and December 2022 were consecutively enrolled in this retrospective study. The inclusion criteria were: (1) was pathologically confirmed as CRC; (2) received tumor resection; (3) had accessible preoperative plasma samples; (4) had available follow-up data. The exclusion criteria were: (1) had any distant metastases (to avoid interference with the prognostic analysis); (2) previously or concurrently had other malignant tumors or hematological diseases; (3) received neoadjuvant therapy (to avoid interference with the level of LRG1); (4) died during the perioperative period (to avoid interference with DFS and OS); (5) with seriously incomplete baseline characteristics (missing data more than 20%). This study was approved by the Ethics Committee of First Hospital of Changsha. All CRC patients or their families submitted informed consent.

Plasma samples collection and LRG1 level detection

The plasma samples of the CRC patients (frozen at −80°C) were obtained and the LRG1 level was measured via enzyme-linked immunosorbent assay (ELISA). The Human LRG1 ELISA Kit (Catalog No. EH10858S, sensitivity: 0.62 ng/mL, range: 3.12-200 ng/mL) was purchased from WeiAO Biotech (Shanghai, China). The experiment was performed strictly according to the instructions of an experimenter unrelated to this study. To better investigate the association between LRG1 level and patient prognosis, the study picked 50 μg/mL (at around the median level of LRG1 in CRC patients) and 100 μg/mL (at around the quartile 3 level of LRG1 in CRC patients) as the cut-off values of LRG1 for analyses.

Data collection and evaluation

The baseline characteristics of CRC patients were extracted from the medical information system. The histological grade was divided into low and high grades based on the 2019 World Health Organization (WHO) classification of tumors of the digestive system (Nagtegaal et al. 2020). The patients' follow-up information was obtained at the same time. Patients underwent normal follow-up with a median period of 1.6 years and a range of 0.2-3.1 years. Based on the patients' follow-up information, the DFS and OS were calculated. Patients who did not experience a DFS or OS event at analysis were censored at their last date of disease assessment.

Data analysis

The statistical analysis software was SPSS 24.0 (IBM SPSS Statistics, Armonk, New York, USA). Mann-Whitney U test was used to compare the LRG1 level in the patients with different age, sex, eastern cooperative oncology group performance status (ECOG PS), histological grade, deficient mismatch repair/microsatellite instability-high (dMMR/MSI-H), abnormal carcinoembryonic antigen (CEA), abnormal carbohydrate antigen 19-9 (CA199), as well as adjuvant chemotherapy. Spearman test was utilized to analyze the correlation of LRG1 level with tumor (T), node (N), and tumor-node-metastasis (TNM) stage. The Kaplan-Meier (K-M) curve displayed the relationship between LRG1 level and patient prognosis, and the lag-rank test was used to analyze. Uni- and multivariate (backward stepwise) Cox regression models were performed to find the factors that would have an association with DFS and OS of the patients. $P < 0.05$ was considered statistically different in this study.

Results

Clinical characteristics

This study enrolled 125 patients with the mean age of 58.0 ± 11.6 years, among whom 61 (48.8%) patients were aged < 60 years and 64 (51.2%) patients were ≥ 60 years. Thirty (24.0%) patients were female and 95 (76.0%) were male. A total of 84 (67.2%) patients were classified as low histological grade and 41 (32.8%) patients were classified as high grade. Regarding TNM stage, there were 23

Table 1. Baseline characteristics of CRC patients.

Characteristics	CRC patients (N = 125)	
	n	%
Age		
< 60 years	61	48.8
≥ 60 years	64	51.2
Sex		
Female	30	24.0
Male	95	76.0
ECOG PS		
0	74	59.2
1	51	40.8
Histological grade		
Low grade	84	67.2
High grade	41	32.8
dMMR/MSI-H	15	12.0
T stage		
1	5	4.0
2	29	23.2
3	84	67.2
4	7	5.6
N stage		
0	69	55.2
1	38	30.4
2	18	14.4
TNM stage		
I	23	18.4
II	46	36.8
III	56	44.8
Abnormal CEA	74	59.2
Abnormal CA199	65	52.0
Adjuvant chemotherapy	79	63.2

CRC, colorectal cancer; ECOG, eastern cooperative oncology group; PS, performance status; dMMR/MSI-H, deficient mismatch repair/microsatellite instability-high; T, tumor; N, node; TNM, tumor-node-metastasis; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9.

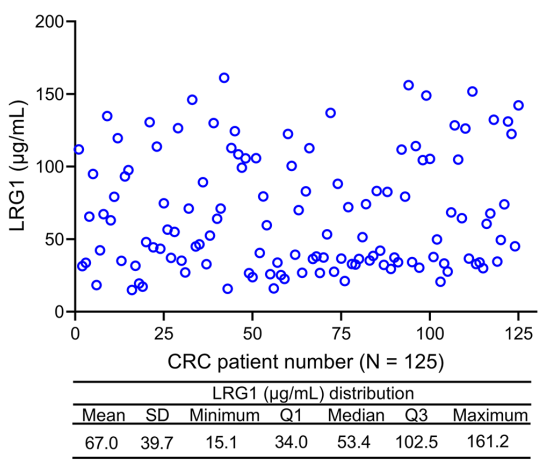


Fig. 1. Level of plasma LRG1 and its distribution in CRC patients who received tumor resection.

Table 2. Correlation between LRG1 and baseline characteristics of CRC patients.

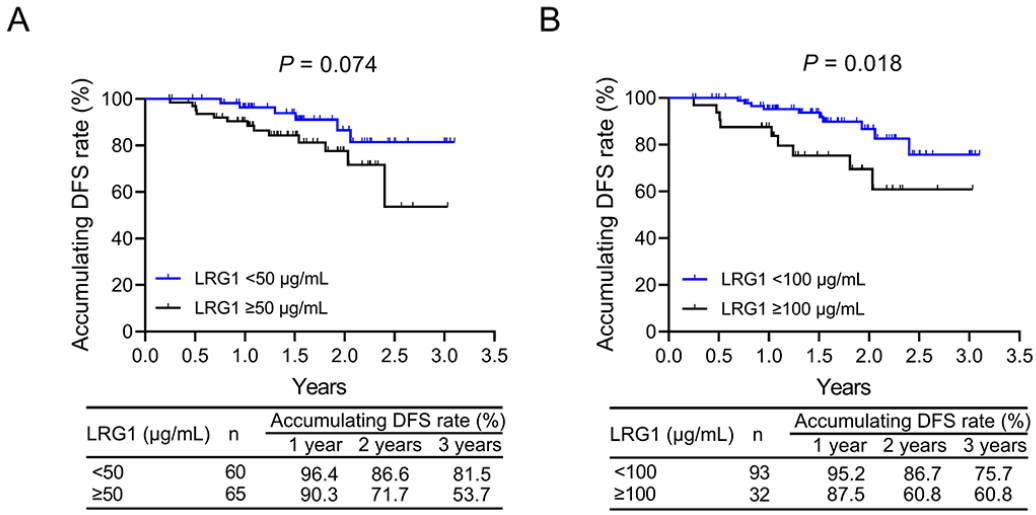
Characteristics	LRG1 (μg/mL)	
	Median (IQR)	*P* value
Age		0.078
< 60 years	42.2 (32.85-102.7)	
≥ 60 years	65.1 (36.6-103.2)	
Sex		0.308
Female	43.0 (33.5-76.9)	
Male	56.6 (34.0-104.9)	
ECOG PS		0.107
0	46.7 (33.2-88.5)	
1	64.5 (35.1-108.5)	
Histological grade		0.005
Low grade	44.0 (32.9-83.2)	
High grade	74.8 (38.2-118.2)	
dMMR/MSI-H		0.226
No	55.8 (34.1-105.5)	
Yes	37.4 (32.9-74.8)	
T stage		0.152
1	38.5 (26.1-56.9)	
2	45.0 (34.0-81.4)	
3	61.8 (34.4-105.6)	
4	35.3 (32.6-134.9)	
N stage		0.004
0	39.4 (32.6-72.7)	
1	85.8 (42.3-116.5)	
2	66.0 (33.9-107.2)	
TNM stage		0.001
I	43.5 (31.5-64.5)	
II	38.8 (32.7-83.3)	
III	82.8 (38.1-112.5)	
Abnormal CEA		0.124
No	46.5 (33.9-79.3)	
Yes	64.1 (33.8-109.3)	
Abnormal CA199		0.264
No	46.6 (34.4-82.9)	
Yes	60.6 (33.1-112.8)	
Adjuvant chemotherapy		0.094
No	44.0 (34.0-75.4)	
Yes	67.8 (33.8-111.8)	

LRG1, leucine-rich alpha-2-glycoprotein 1; CRC, colorectal cancer; IQR, interquartile range; ECOG, eastern cooperative oncology group; PS, performance status; dMMR/MSI-H, deficient mismatch repair/microsatellite instability-high; T, tumor; N, node; TNM, tumor-node-metastasis; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 19-9.

(18.4%), 46 (36.8%), and 56 (44.8%) patients at stage I, II, and III, accordingly. In addition, 79 (63.2%) patients were treated with adjuvant chemotherapy. The detailed characteristics of patients are listed in Table 1.

Plasma LRG1 distribution

The level of plasma LRG1 in each patient is shown in



LRG1 (μg/mL)	n	Accumulating DFS rate (%)		
		1 year	2 years	3 years
<50	60	96.4	86.6	81.5
≥50	65	90.3	71.7	53.7

LRG1 (μg/mL)	n	Accumulating DFS rate (%)		
		1 year	2 years	3 years
<100	93	95.2	86.7	75.7
≥100	32	87.5	60.8	60.8

LRG1 (μg/mL)	n	Accumulating OS rate (%)		
		1 year	2 years	3 years
<50	60	100.0	92.3	86.9
≥50	65	98.3	83.6	62.1

LRG1 (μg/mL)	n	Accumulating OS rate (%)		
		1 year	2 years	3 years
<100	93	100.0	93.1	82.9
≥100	32	96.9	73.6	64.4

Fig. 2. Plasma LRG1 ≥ 100 μg/mL was associated with shortened DFS and OS in CRC patients who received tumor resection. Association of plasma LRG1 ≥ 50 μg/mL (A) and ≥ 100 μg/mL (B) with DFS in CRC patients who received tumor resection. Association of LRG1 ≥ 50 μg/mL (C) and ≥ 100 μg/mL (D) with OS in CRC patients who received tumor resection.

Fig. 1. The mean level of plasma LRG1 was 67.0 ± 39.7 μg/mL. The median level of plasma LRG1 was 53.4 μg/mL (quartile 1: 34.0 μg/mL, quartile 3: 102.5 μg/mL). In addition, the minimum and maximum level of plasma LRG1 was 15.1 μg/mL and 161.2 μg/mL, correspondingly.

Comparison of plasma LRG1 among patients with different clinical characteristics

Plasma LRG1 was elevated in patients with high histological grade compared to those with low grade ($P = 0.005$). Plasma LRG1 was varied among patients with different N stages ($P = 0.004$) and TNM stages ($P = 0.001$). However, plasma LRG1 was not varied in patients with different ages, sexs, ECOG PS scores, dMMR/MSI-H status, T stages, CEA levels, or CA199 levels, or adjuvant therapy status (all $P > 0.050$) (Table 2).

Association of plasma LRG1 with DFS and OS

Plasma LRG1 ≥ 50 μg/mL was not linked with DFS ($P = 0.074$). The 3-year accumulating DFS rate was 53.7% and 81.5% in patients with plasma LRG1 ≥ 50 μg/mL and < 50 μg/mL, respectively (Fig. 2A). Plasma LRG1 ≥ 100 μg/mL was related to shortened DFS ($P = 0.018$). The 3-year accumulating DFS rate was 60.8% and 75.7% in patients with plasma LRG1 ≥ 100 μg/mL and < 100 μg/mL, correspondingly (Fig. 2B). Plasma LRG1 ≥ 50 μg/mL was not associated with OS ($P = 0.077$). The 3-year accumulating OS rate was 62.1% in patients with plasma LRG1 ≥ 50 μg/mL and 86.9% in those with plasma LRG1 < 50 μg/mL (Fig. 2C). While plasma LRG1 ≥ 100 μg/mL was linked with shortened OS ($P = 0.016$). The 3-year accumulating OS rate was 64.4% and 82.9% in patients with plasma LRG1 ≥ 100 μg/mL and < 100 μg/mL, accordingly (Fig. 2D).

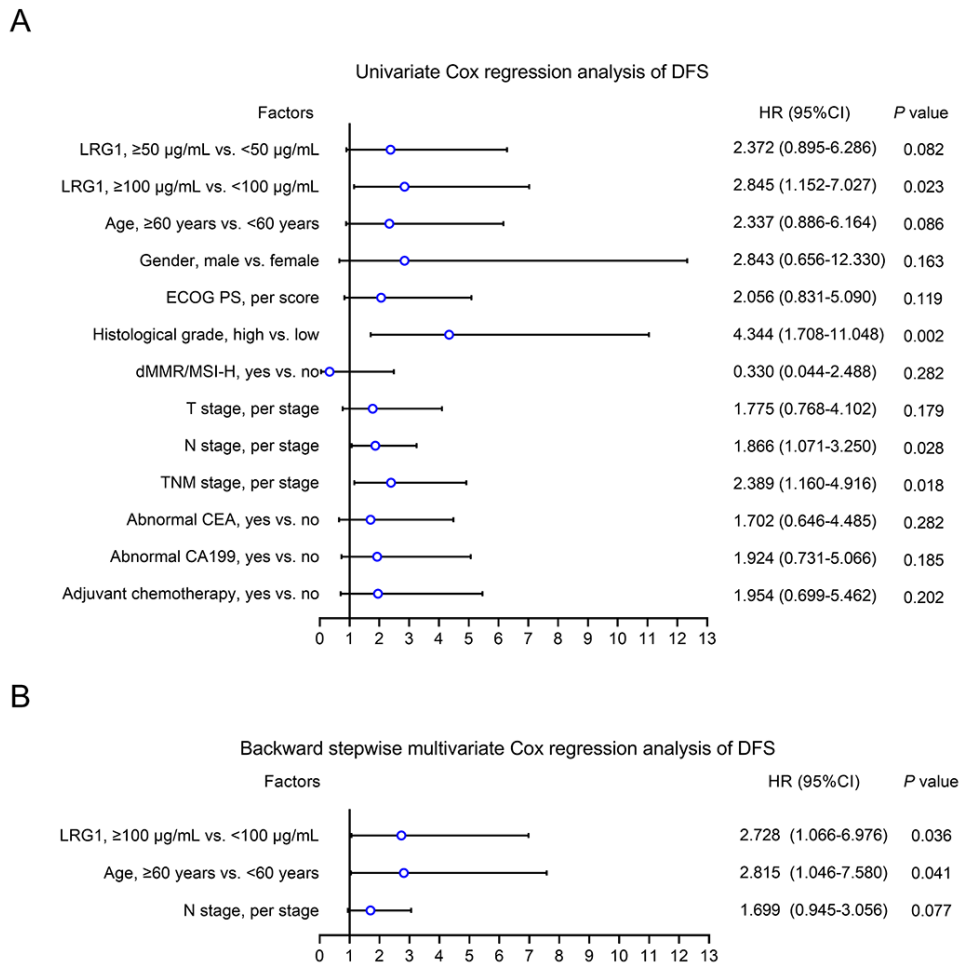


Fig. 3. Plasma LRG1 ≥ 100 μg/mL was independently associated with shortened DFS in CRC patients who received tumor resection.
Univariate (A) and multivariate (backward stepwise) (B) Cox regression models for DFS in CRC patients who received tumor resection.

Related factors of DFS

Plasma LRG1 (≥ 100 μg/mL vs. < 100 μg/mL) (P = 0.023), histological grade (high vs. low) (P = 0.002), N stage (per stage) (P = 0.028), and TNM stage (per stage) (P = 0.018) were linked with worse DFS (Fig. 3A). After adjustment, plasma LRG1 (≥ 100 μg/mL vs. < 100 μg/mL) (hazard ratio (HR): 2.728, 95% confidence interval (CI): 1.066-6.976, P = 0.036) and age (≥ 60 years vs. < 60 years) (HR: 2.815, 95% CI: 1.046-7.580, P = 0.041) were independently associated with shortened DFS (Fig. 3B).

Related factors of OS

Plasma LRG1 (≥ 100 μg/mL vs. < 100 μg/mL) (P = 0.026), histological grade (high vs. low) (P = 0.007), N stage (per stage) (P = 0.004), and TNM stage (per stage) (P = 0.031) were related to worse OS (Fig. 4A). Furthermore, only N stage (per stage) (HR: 3.150, 95% CI: 1.450-6.842, P = 0.004) was independently linked with shortened OS (Fig. 4B).

Discussion

LRG1 participates in the progression of many cancers through regulating angiogenesis, migration, EMT, and apoptosis of cancer cells (Lin et al. 2022). Clinically, some studies have reported the linkage of LRG1 with tumor characteristics in cancer patients (Wang et al. 2015; Sun et al. 2017; Zhang et al. 2021). For instance, one study reveals that LRG1 is associated with worse tumor differentiation in stage III CRC patients (Sun et al. 2017). Another study shows that LRG1 is related to increased TNM stage in breast cancer patients (Zhang et al. 2021). In this study, plasma LRG1 was linked with elevated histological grade in CRC patients who received tumor resection. The probable explanation could be: LRG1 was associated with phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha mutation in solid tumor, and the latter was related to poor tumor differentiation in CRC (Ramirez-Ardila et al. 2016; Zeng et al. 2023). Also, this study found that plasma LRG1 showed a positive trend to associate with N stage in

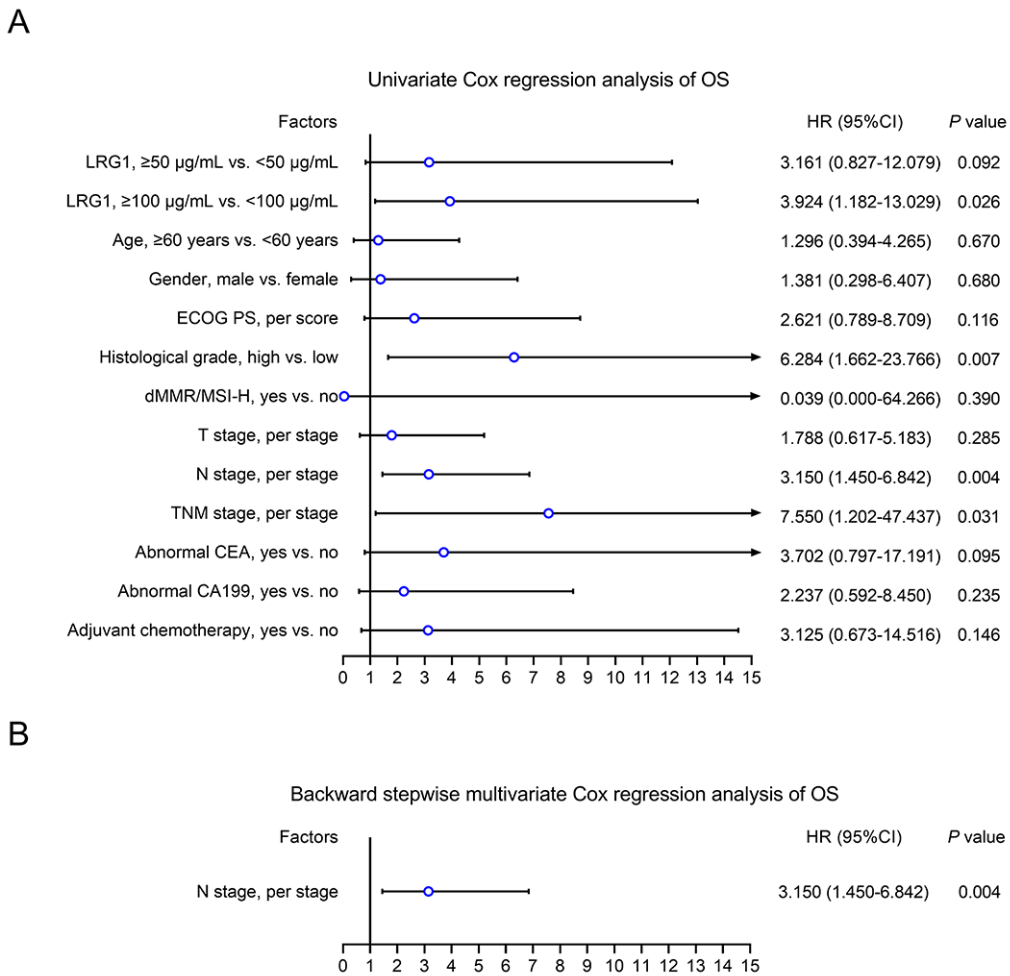


Fig. 4. Plasma LRG1 was not independently associated with shortened OS in CRC patients who received tumor resection. Univariate (A) and multivariate (backward stepwise) (B) Cox regression models for OS in CRC patients who received tumor resection.

CRC patients who received tumor resection. The possible reason could be: LRG1 increased CRC cell migration and invasion (Zhong et al. 2021), thereby, its plasma level was associated with N stage in CRC patients who received tumor resection. In addition, this study also observed the relation between plasma LRG1 and TNM stage in CRC patients who received tumor resection, which might be contributed to the association of plasma LRG1 with N stage in these patients. Nevertheless, the linkage of plasma LRG1 with clinical stage in CRC patients who received tumor resection required more exploration.

More importantly, previous studies have showed that increased LRG1 is associated with worse survival outcomes in cancer patients (Sun et al. 2017; Wang et al. 2019; Zhang et al. 2021). For example, LRG1 is an independent factor for shortened DFS and OS in stage III CRC patients (Sun et al. 2017). In another study, LRG1 is related to worse progression-free survival and OS in patients with esophageal squamous cell carcinoma (Wang et al. 2019). Regarding CRC patients who received tumor resection, this study set 50 μg/mL (at around the median level) and 100 μg/mL (at around the quartile 3 level) as two cutoff values of plasma LRG1 to evaluate its prognostic value in these patients. The data revealed that plasma LRG1 $\geq$ 100 μg/mL was linked with shortened DFS and OS in these patients. Moreover, plasma LRG1 $\geq$ 100 μg/mL was independently linked with shortened DFS in CRC patients who received tumor resection. The probable explanations could be: (1) LRG1 regulated CRC migration, invasion, EMT, and metastases through related proteins, such as TGF-β and hypoxia-inducible factor-1α, which reflected the severity of CRC (Zhang et al. 2016; Zhong et al. 2021; Zou et al. 2021; Chan et al. 2022). (2) LRG1 affected the tumor microenvironment, especially immunity, by destabilizing tumor vessels, inhibiting T lymphocyte infiltration, and enhancing immunosuppression, which subsequently influenced the prognoses of CRC patients (den Uil et al. 2019; Lamplugh and Fan 2021; O'Connor et al. 2021). Thus, LRG1 $\geq$ 100 μg/mL reflected worse DFS and OS in CRC patients who received tumor resection. Whereas abnormal CEA and CA199, the most common tumor biomarkers for predicting prognosis, were not related to DFS or OS in CRC patients

who received tumor resection according to univariate Cox regression analyses in this study. The HRs for DFS were 1.702 for CEA and 1.924 for CA199; the HRs for OS were 3.702 for CEA and 2.237 for CA199 in these patients. All these values were lower than the HRs of LRG1 ≥ 100 μg/mL, which were 2.845 for DFS and 3.924 for OS in these patients. These findings indicated that LRG1 might have the potential for predicting prognoses in CRC patients undergoing tumor resection, while its prognostic value compared to other common blood markers (such as CEA and CA199) needed more studies for validation. In addition, this study also found that age ≥ 60 years was independently related to shortened DFS, and N stage was independently linked with shortened OS in CRC patients who received tumor resection, which was consistent with previous studies (Tang et al. 2020; Li et al. 2022; Quan et al. 2023; Seo and Park 2023).

In this study, LRG1 level was detected in blood samples from CRC patients before surgery, but not tumor tissues. The reason was that: blood samples are easier and less invasive to obtain compared to tumor tissue samples, which allows for wider application of plasma LRG1 as a prognostic noninvasive biomarker in clinical practice. Additionally, CRC patients who received neoadjuvant therapy were excluded to avoid interference with the level of LRG1. However, blood LRG1 in CRC patients who receive tumor resection before and after neoadjuvant therapy is uncertain, and needs further studies for validation.

Some limitations still existed in the present study. First, the sample size of this study was relatively small, which weakened the statistical power. Second, this study did not enroll healthy subjects. Thereby, the question of whether the level of LRG1 in CRC patients who received tumor resection was abnormal compared to healthy subjects remained unanswered. Third, this was a retrospective study and patients who did not have blood sample collection were excluded, which led to selection bias. Fourth, this study did not collect postoperative plasma samples from CRC patients who received tumor resection, thereby, the change of LRG1 after surgery was unable to be analyzed, which should be detected in further studies. Finally, the follow-up period was relatively short in this study (median: 1.6 years, range: 0.2-3.1 years) and most patients did not reach endpoint events; thus, investigations with longer follow-up duration are needed to validate the ability of LRG1 for predicting survival in CRC patients who received tumor resection.

In summary, plasma LRG1 is associated with elevated histological grade and worse DFS and OS, whose level ≥ 100 μg/mL is independently linked with shortened DFS in CRC patients who receive tumor resection. These findings suggest that LRG1 owns potential in identifying patients with unsatisfactory outcomes, which may be a useful molecular tool for precision medicine in CRC.

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Conflict of Interest

The authors declare no conflict of interest.

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