Clinicopathological significance and impact on the outcomes of IGF-1R and Livin in patients with colorectal cancer

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Research Article

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Abstract

Background: Colorectal cancer (CRC) is the third most common cancer worldwide. However, limited effective biomarkers are associated with the tumorigenesis and prognosis of CRC.

Methods: The present study identified potential signatures from The Cancer Genome Atlas (TCGA) database and further validated the identified biomarkers in CRC tissues by immunohistochemistry (IHC).

Results: The expression of insulin-like growth factor 1 receptor (*IGF-1R*) and *Livin* gene was significantly upregulated in CRC samples compared to the adjacent normal samples in TCGA dataset. IHC indicated that IGF-1R and Livin protein levels are increased in CRC and adenoma tissues compared to normal tissues. Notably, the IGF-1R protein levels differed significantly between adenoma and CRC. The elevated IGF-1R and Livin expressions were associated with CRC clinicopathological features, including age, gender, histological subtype, individual cancer stages, nodal metastasis, and TP53-mutant in TCGA. Additionally, IGF-1R promoter methylation level was closely related to CRC. Consistent with the TCGA study, IHC indicated that overexpressed IGF-1R and Livin proteins were independent risk factors for stage and metastasis. A marked correlation was established between IGF-1R and Livin expression in CRC, while the survival map showed no significant correlation with CRC. Kaplan-Meier survival curves showed that CRC patients with high IGF-1R or Livin expression had longer overall survival and disease-free survival than those with low expression in TCGA.

Conclusion: IGF-1R and Livin are associated with CRC tumorigenesis and might be valuable for novel biomarker identification and targeted therapeutic strategy development.

Introduction

Colorectal cancer (CRC) is the third most common malignant tumor and the second most common cause of death worldwide. According to the GLOBOCAN project of the World Health Organization (WHO) Cancer Research Center, the number of new CRC cases in 2020 is about 1.93 million worldwide, and the number of deaths is about 940,000 [1]. It is estimated that by 2030, the global burden of CRC will increase by 60%: the number of new cases will exceed 2.2 million, and the number of deaths will exceed 1.1 million [2]. Several risk factors, including age, hereditary components, chronic intestinal inflammation, obesity, excessive alcohol and red meat consumption, smoking, and lack of physical exercise, have been identified for CRC [3-5]. Despite the advancement in comprehensive therapy, the long-term survival of CRC patients remains unsatisfactory [6, 7]. The poor therapeutic outcome of patients with CRC is mainly due to local recurrence and distant metastasis [8]. Therefore, understanding the pathogenesis of CRC and finding effective therapeutic targets is imperative.

Insulin-like growth factor 1 receptor (IGF-1R) belongs to the tyrosine kinase receptor family. The elevated expression and activity of IGF-1R is observed in many cancer types and is associated with tumor cell proliferation, survival, anti-apoptosis, and drug resistance [9-11]. IGF-1R triggers various intracellular signaling cascades in colonic mucosal cells that enhance cell cycle progression and inhibit apoptosis

[12]. IGF-1R inhibition results in the G1 cell cycle arrest and a significant decrease in CRC cell proliferation, survival, and radioresistance [13, 14]. In a previous study, we demonstrated that IGF-1R is overexpressed in HT-29 cell lines. Cyclolignan picropodophyllin (PPP, an IGF-1R inhibitor) inhibits the proliferation and migration of HT-29 cell lines in a dose-dependent manner and decreases expression/ phosphorylation of IGF-1R, extracellular signal-regulated kinase 1/2 (ERK1/2), and Akt [15]. The overexpression of the inhibitor of apoptosis protein (IAP) family, including baculoviral IAP repeat-containing protein-7 (BIRC7), facilitates apoptosis evasion in CRC [16]. BIRC7 is also referred to as Livin or melanoma inhibitor of apoptosis. Livin potentiates migration and invasion of CRC cells partially via nuclear factor-kappa B (NF-κB)-mediated epithelial-mesenchymal transition [17]. Although both IGF-1R and Livin have been reported as potential biomarkers for CRC, only a few studies have investigated their role in the progression of normal mucosa-colorectal adenoma-colorectal cancer.

The analysis of differential gene expression between diverse cancer types and adjacent normal tissues across The Cancer Genome Atlas (TCGA) cohort revealed higher *IGF-1R* and *Livin* gene expression in patients with colon adenocarcinoma (COAD) compared to the adjacent normal tissues from the Sangerbox, GEPIA2, and UALCAN portal datasets. We also used TCGA-COAD to explore the clinical significance, patient survival, and correlation between IGF-1R and Livin expression and CRC. In agreement with the TCGA study, we selected CRC (n = 60), colorectal adenoma (n = 30), and adjacent normal tissues (n = 10) and their clinicopathological data, detected the expression and distribution of IGF-1R and Livin proteins in various intestinal mucosal tissues and analyzed the correlation between IGF-1R and Livin expression and clinicopathological parameters, occurrence, development, and clinical prognosis of CRC patients. These data may reveal a novel oncogenic function and the clinical value of IGF-1R and Livin in CRC.

Materials And Methods

Patients and surgical specimens

A total of 60 patients with primary CRC who underwent surgical resection at the General Surgery Department of the Second Affiliated Hospital of Dalian Medical University (Dalian, China) comprised the CRC group. The cohort was in Dukes' stages A, B, C, and D (n = 15 cases). None of the patients had received chemotherapy, radiotherapy, or other anti-cancer treatment before the operation. Biopsies from 10 cases of adjacent non-tumor tissues comprised the control group. 30 patients who underwent colonoscopy and endoscopic polypectomy, endoscopic mucosal resection (EMR), or endoscopic submucosal dissection (ESD) at the Department of Gastroenterology of the Second Affiliated Hospital of Dalian Medical University were regarded as the colorectal adenoma group, including 8 cases of tubular adenoma and 22 cases of villous tubular adenoma. All tissue specimens were confirmed by pathology, and all the clinicopathological data were complete. Samples from patients with diabetes, acromegaly, dwarfism, cachexia, severe organ failure, or other malignant tumors were excluded. This research protocol has been quick reviewed by the Ethics Committee of the Second Affiliated Hospital of Dalian Medical

University and agreed the project will carry out on the premise of protecting the rights and interests of subjects.

Data processing and analysis

For gene expression, clinical significance, and survival analysis, the TCGA-COAD datasets from Sangerbox (http://sangerbox.com/Index), GEPIA2 (http://gepia2.cancer-pku.cn/#analysis), and UALCAN portal (http://ualcan.path.uab. edu/analysis-prot.html) were used. The significance of difference was estimated by Student's t-test considering unequal variance for gene expression and clinical significance analysis. The normalized average counts from the adjacent normal tissue samples served as the threshold to filter the samples. Then, only samples with counts above the threshold were used for survival analysis. Survival plots were drawn by comparing the top 30% (high-expression group) and the bottom 30% filtered samples (low-expression group). The P-values were calculated by log-rank test.

The correlation analysis was performed with the GEPIA2 dataset in the category "colorectal adenocarcinoma" and "adjacent normal tissues" from TCGA cohort, using the non-log scale for calculation and the log-scale axis for visualization.

Immunohistochemistry (IHC)

The specimens were fixed in formalin and embedded with paraffin before slicing into 3-μm-thick sections. After deparaffinization using xylene and dehydration using graded ethanol, the sections were immersed into citrate buffer at pH 7.2-7.4 for antigen retrieval. Then, the sections were probed with IGF-1R (1:200; Abcam, Cambridge, UK) and Livin (1:200; Proteintech, Shanghai, China) antibodies at 4 °C overnight and 37 °C for 1 h, followed by phosphate-buffered saline (PBS) washes. Subsequently, the sections were incubated with a biotinylated secondary antibody (Zhongshan Golden Bridge Biotech Co. Ltd, Beijing, China) at 37 °C for 30 min. The immunoreaction was developed by incubation with diaminobenzidine (DAB) for 10 min. Hematoxylin was used for counterstaining and alcohol gradient for dehydration. Finally, the sections were sealed with neutral gum and observed under a light microscope (Olympus Bx-51, Japan). All the slides were examined by an experienced pathologist blinded to the study design.

Staining results: IGF-1R and Livin were observed in the cell membrane as yellow or brownish-yellow staining. Five random fields were examined at high magnification for each sample. The staining intensity (no staining is 0, slight staining is 1 point, moderate staining is 2 points, and deep staining is 3 points) and ratios of positive cells (0-25% refers to 0 points, 26-50% is 1 point, 51-75% is 2 points, > 76% is 3 points) were determined using the couple score and semiquantitative method. The final results were multiplied by two scores mentioned above: 0-1 point, negative (−); 2-4 points, weak positive (+); 5-7 points, positive (++); 8-9 points, strong positive (+++).

Statistical analysis

The data were analyzed using SPSS 20.0 statistical software (IBM Co., Armonk, NY, USA). Mann-Whitney U test or Kruskal-Wallis test was used for enumeration data, and Spearman's chi-squared test was used

for correlation analysis. The test standard $\alpha = 0.05$ and $P < 0.05$ indicated significant difference.

Results

IGF-1R is frequently overexpressed in primary CRC tumors

The analysis of the *IGF-1R* gene expression between diverse cancer types and adjacent normal tissues across The Cancer Genome Atlas (TCGA) cohort revealed high gene expression in COAD (n = 458) compared to the adjacent normal tissues (n = 41) ($P < 0.05$, Fig. 1A) from Sangerbox dataset. Similarly, upregulated *IGF-1R* gene expression was also observed in primary COAD in GEPIA2 dataset (n = 275, Fig. 1B) and UALCAN portal dataset (n = 286, $P < 0.05$, Fig. 1C). Furthermore, the IHC results showed significantly higher levels of IGF-1R in tubular adenoma, villous adenoma, and CRC tissues compared to the adjacent normal tissues, as assessed by the Mann-Whitney U test, and the difference between adenoma and CRCs was statistically significant (Fig. 1D, Table 1). These results demonstrated that IGF-1R is commonly overexpressed in CRC and may play a critical role in the growth and malignant transformation of precancerous polyps.

Livin expression is upregulated in CRC

Next, we analyzed the Livin (also known as BIRC7) expression in primary CRC tissues from TCGA cohort. The gene expression was significantly upregulated in primary COAD compared to the adjacent normal tissues in the Sangerbox dataset ($P < 0.001$, Fig. 2A). Specifically, the upregulation of *Livin* gene expression in COAD was respectively validated in two datasets (GEPIA2 and UALCAN) of paired tumor and adjacent normal samples from TCGA study (Fig. 2B and 2C). To further analyze Livin expression and validate these findings, we conducted Livin IHC in a cohort of 100 patients with localized CRCs, adenoma, and adjacent normal tissues. The staining showed that the level of Livin protein in the colorectal adenoma and cancer groups was significantly higher than that of the adjacent normal tissues; however, no significant difference was detected between the colorectal adenoma and cancer groups (Fig. 2D, Table 1). These results demonstrated that the high level of Livin protein is related to the occurrence of CRC, and it may not be a crucial factor in the progression of adenoma to CRC.

Overexpression of IGF-1R is an independent predictor of oncogenic function in CRC patients

We further evaluated the clinicopathological significance of the IGF-1R gene and protein expression in CRC patients. A significant correlation was established between *IGF-1R* gene expression and clinicopathologic features, including age (61-80 years), gender (male), histological subtype (adenocarcinoma), individual cancer stages (stage 3), nodal metastasis status (N1 and N2), and TP53-mutant (Fig. 3A-E and 3G). In addition, the promoter methylation level of IGF-1R was closely related to COAD. We also evaluated the correlation between IGF-1R protein expression and clinicopathological features in colorectal cancer. Consistent with the TCGA study, IGF-1R protein overexpression was associated with the depth of invasion, Dukes' stage, lymph node metastasis, and distant metastasis in patients with CRC (all $P < 0.05$, Table 2), and with the increase in Dukes' stage, the expression level of IGF-

1R protein increased significantly (Fig. 3H, Table 2). However, no correlation was established between IGF-1R protein expression and age, gender, diameter, location, histological subtype, and differentiation (Table 2). These results demonstrated that IGF-1R might be used as a biomarker to evaluate the condition of CRC patients.

Clinical application value of Livin expression in CRC

We also analyzed the association between Livin gene and protein expression and the clinicopathological features of CRC. As shown in Fig. 4A-G, a significant correlation was established between *Livin* gene expression and clinicopathological features, including age (41-60, 61-80, and 81-100 years), gender (male and female), histological subtype (adenocarcinoma), individual cancer stages (1, 2, and 3), nodal metastasis status (N0, N1, and N2), and TP53 mutation status (TP53-mutant and TP53-non-mutant), while no correlation was established in the promoter methylation level of Livin. Moreover, Spearson's chi-square test analysis showed that Livin protein overexpression was an independent risk factor for stage and metastasis, but was not associated with age, gender, tumor size, primary tumor location, pathological type, histological type, or depth of invasion (Fig. 4H, Table 2).

IGF-1R and Livin are not prognostic markers of CRC

The correlation between IGF-1R and Livin expression and patients' overall survival (OS) and disease-free survival (DFS) was estimated by Kaplan-Meier analysis. The survival map showed that both IGF-1R and Livin are not significantly correlated with COAD from TCGA dataset (Fig. 5A and 5B). We sorted the patient cases from TCGA-COAD with survival data into the high- and low-expression of IGF-1R and Livin groups. As shown in the Kaplan-Meier survival curves, CRC patients with high IGF-1R or Livin expression showed a longer OS and DFS than those with low expression based on the log-rank test ($P > 0.05$ for both OS and DFS, Fig. 5C-F). Collectively, these findings indicated that neither IGF-1R nor Livin serve as an independent new biomarker for the prognosis of CRC patients. Moreover, a marked correlation was established between IGF-1R and Livin expression in COAD by Spearman's chi-squared test analysis ($R = 0.2$, $P = 0.00048$, Fig. 6).

Discussion

In this study, we found that *IGF-1R* and *Livin* genes are highly expressed in CRC and the other cancer types, such as cholangiocarcinoma and kidney, liver, and lung cancers, through TCGA database [18-21]. Also, the expression of *IGF-1R* and *Livin* genes was increased in the 275 CRC cases from the GEPIA2 dataset and 286 colorectal cancer cases from the UALCAN portal dataset. IGF-1R may play a role in the neoplastic progression of CRC because it is related to both cellular proliferation and differentiation [22]. The expression of Livin, a member of the inhibitors of the apoptosis protein family, is associated with tumor development and progression [23]. The results of IHC showed a significant correlation between the expression of IGF-1R protein and neoplastic progression from normal mucosa to adenomatous polyps and finally to CRC. The high expression of Livin protein is related to the occurrence of CRC, but it may not be a critical factor in the progression of adenoma to CRC.

We further investigated the clinical importance of IGF-1R and Livin expression in CRC patients at mRNA and protein levels in two independent cohorts and found that both molecules were overexpressed. According to the TCGA study, the levels of IGF-1R and Livin were associated significantly with age, gender, histological subtype, individual cancer stages, nodal metastasis status, and TP53 mutation status in CRC relative to the adjacent normal tissues. *TP53* is a well-known tumor suppressor gene that encodes p53 and is frequently inactivated by mutation or deletion in most human CRCs [24, 25]. However, no correlation was established between IGF-1R and Livin protein expression and clinicopathological features, such as age, gender, diameter, location, differentiation, and histological subtype from 60 patients with CRC. Both the TCGA cohort and our results showed that high levels of IGF-1R and Livin were significantly correlated with stage and metastasis. Moreover, the promoter methylation level of IGF-1R, but not Livin, was closely related to CRC.

Both survival map and Kaplan-Meier survival curves showed that high expression levels of IGF-1R and Livin were not correlated significantly with the short OS of patients with CRC. The clinical outcome of CRC varies greatly depending on the aggressiveness of individual tumors. Many patients experience disease recurrence following radical surgery. Nonetheless, the TCGA dataset demonstrated that neither IGF-1R nor Livin serves as an independent prognostic marker for CRC patients, which was opposite to the previous findings. Thus, additional prognostic biomarkers may accurately assess the risk to guide personalized chemotherapy. Interestingly, these results showed a marked correlation between IGF-1R and Livin expression in COAD by Spearman's chi-square test. In the future, we will use confocal fluorescence staining to study the co-localization of IGF-1R and Livin proteins in CRC tissues to determine whether double-positive expression promotes the occurrence and development of CRC more than single-positive expression.

Conclusions

In summary, we demonstrated that IGF-1R and Livin are highly expressed oncogenes in CRC patients. Both can be considered biomarkers for the stage, metastasis, and risk of carcinogenesis. IGF-1R may drive neoplastic initiation and progression along the colorectal normal mucosa-polyp-cancer sequence. In addition, our findings indicated that IGF-1R and Livin are not independent prognostic markers for patients with CRC. Nonetheless, further investigations are needed to substantiate our findings.

Abbreviations

CRC: Colorectal cancer; IGF-1R: Insulin-like growth factor 1 receptor; BIRC7: Baculoviral IAP repeat-containing protein-7; COAD: Colon adenocarcinoma; TCGA: The Cancer Genome Atlas; IHC: Immunohistochemistry; OS: Overall survival; DFS: Disease-free survival.

Declarations

Acknowledgements

Not applicable.

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Availability of data and materials

The datasets analysed during the current study are available from The Cancer Genome Atlas (TCGA) database, the web links are Sangerbox (http://sangerbox.com/Index), GEPIA2 (http://gepia2.cancer-pku.cn/#analysis), and UALCAN portal (http://ualcan.path.uab. edu/analysis-prot.html). Other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Feng XY, Zhang ZL and Zhang YX conceived the project, designed and supervised this study. Zhang ZL and Pan ZA carried out the patient samples experiment. Zhang ZL and Lao S performed the data analysis. Zhang ZL, Qiu J and Feng XY wrote and edited manuscript. All authors approved the final version of the manuscript.

Ethics approval

All experiments were performed in accordance with relevant guidelines and regulations. This research protocol has been quick reviewed by the Ethics Committee of the Second Affiliated Hospital of Dalian Medical University and agreed the project will carry out on the premise of protecting the rights and interests of subjects. The study is a retrospective study, the patient has not been contacted more than 3 times, and the research project does not involve personal privacy or commercial interests, so we apply for exemption from signing the informed consent.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no conflict of interests.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA-Cancer J Clin. 2021; 71: 209-249.

2. Arnold M, Sierra MS, Laversanne M, et al. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017; 66: 683-691.
3. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol. 2019; 16: 713-732.
4. Wijnands AM, de Jong ME, Lutgens MWMD, et al. Prognostic factors for advanced colorectal neoplasia in inflammatory bowel disease: systematic review and meta-analysis. Gastroenterology. 2021; 160: 1584-1598.
5. Chen H, Zheng X, Zong X, et al. Metabolic syndrome, metabolic comorbid conditions and risk of early-onset colorectal cancer. Gut. 2021; 70: 1147-1154.
6. van Lanschot MCJ, van Leerdam ME, Lansdorp-Vogelaar I, et al. Yield of surveillance colonoscopies 1 year after curative surgical colorectal cancer resections. Clin Gastroenterol Hepatol. 2019; 17: 2285-2293.
7. Guraya SY. Pattern, stage, and time of recurrent colorectal cancer after curative surgery. Clin. Colorectal. Cancer. 2019; 18: e223-e228.
8. Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. Lancet. 2019; 394: 1467-1480.
9. Muoio MG, Talia M, Lappano R, et al. Activation of the S100A7/RAGE pathway by IGF-1 contributes to angiogenesis in breast cancer. Cancers (Basel). 2021; 13: 621.
10. Fuentes-Baile M, Ventero MP, Encinar JA, et al. Differential effects of IGF-1R small molecule tyrosine kinase inhibitors BMS-754807 and OSI-906 on human cancer cell lines. Cancers (Basel). 2020; 12: 3717.
11. Wang F, Zhang L, Sai B, et al. BMSC-derived leptin and IGFBP2 promote erlotinib resistance in lung adenocarcinoma cells through IGF-1R activation in hypoxic environment. Cancer Biol Ther. 2020; 21: 61-71.
12. Wang SQ, Yang XY, Yu XF, et al. Knockdown of IGF-1R triggers viral RNA sensor MDA5- and RIG-I-mediated mitochondrial apoptosis in colonic cancer cells. Mol Ther Nucleic Acids. 2019; 16: 105-117.
13. Samadi P, Afshar S, Amini R, et al. Let-7e enhances the radiosensitivity of colorectal cancer cells by directly targeting insulin-like growth factor 1 receptor. J Cell Physiol. 2019; 234: 10718-10725.
14. Wang Q, Zhang Y, Zhu J, et al. IGF-1R inhibition induces MEK phosphorylation to promote survival in colon carcinomas. Signal Transduct Target Ther. 2020; 5: 153.
15. Feng X, Aleem E, Lin Y, et al. Multiple antitumor effects of picropodophyllin in colon carcinoma cell lines: clinical implications. Int J Oncol.2012; 40: 1251-1258.
16. Güllülü Ö, Hehlgans S, Rödel C, et al. Tumor suppressor protein p53 and inhibitor of apoptosis proteins in colorectal cancer-a promising signaling network for therapeutic interventions. Cancers (Basel). 2021; 13: 624.
17. Ge Y, Cao X, Wang D, et al. Overexpression of Livin promotes migration and invasion of colorectal cancer cells by induction of epithelial-mesenchymal transition via NF-κB activation. Onco Targets Ther. 2016; 9: 1011-1021.

18. Alvaro D, Barbaro B, Franchitto A, et al. Estrogens and insulin-like growth factor 1 modulate neoplastic cell growth in human cholangiocarcinoma. Am J Pathol. 2006; 169: 877-888.
19. Yu H, Liu J, Zhang Z, et al. SNHG12 promotes carcinogenesis of human renal cell cancer via functioning as a competing endogenous RNA and sponging miR-30a-3p. J Cell Mol Med. 2021; 25: 4696-4708.
20. Zhang Q, Li T, Wang Z, et al. lncRNA NR2F1-AS1 promotes breast cancer angiogenesis through activating IGF-1/IGF-1R/ERK pathway. J Cell Mol Med. 2020; 24: 8236-8247.
21. Wang R, Yamada T, Kita K, et al. Transient IGF-1R inhibition combined with osimertinib eradicates AXL-low expressing EGFR mutated lung cancer. Nat Commun. 2020; 11: 4607.
22. Shan HB, Zhang R, Li Y, et al. Expression of IGF-1R in colorectal polyps and its role in colorectal carcinogenesis. Technol Cancer Res Treat.2011; 10: 381-389.
23. Myung DS, Park YL, Chung CY, et al. Expression of Livin in colorectal cancer and its relationship to tumor cell behavior and prognosis. PLoS One. 2013; 8: e73262.
24. Terradas M, Mur P, Belhadj S, et al. TP53, a gene for colorectal cancer predisposition in the absence of Li-Fraumeni-associated phenotypes. Gut. 2021; 70: 1139-1146.
25. Kim JE, Choi J, Sung CO, et al. High prevalence of TP53 loss and whole-genome doubling in early-onset colorectal cancer. Exp Mol Med. 2021; 53: 446-456.

Tables

Table 1-2 are available in the Supplementary Files section.

Figures

Figure 1

IGF-1R expression in CRC patients. *IGF-1R* gene expression between diverse cancer types (A) and primary COAD (B and C) from TCGA database. (D) IGF-1R protein expression in paracancerous, tubular adenoma, villous adenoma, and CRC patients tissues by IHC staining (400×, scale = 10 μm). IGF-1R, insulin-like growth factor 1 receptor; COAD, colorectal adenocarcinoma; TCGA, The Cancer Genome Atlas; CRC, colorectal cancer; IHC, immunohistochemistry. *P < 0.05, **P < 0.01, and ***P < 0.001.

Figure 2

Livin expression in CRC patients. *Livin* gene expression between diverse cancer types (A) and primary COAD (B and C) from TCGA database. (D) Livin protein expression in paracancerous, tubular adenoma,

villous adenoma, and CRC patients tissues by IHC staining (400×, scale = 10 μm). COAD, colorectal adenocarcinoma; TCGA, The Cancer Genome Atlas; CRC, colorectal cancer; IHC, immunohistochemistry. *P < 0.05, **P < 0.01, and ***P < 0.001.

Figure 3

Elevated IGF-1R expression is associated with the tumorigenesis and progression of CRC. The correlation between *IGF-1R* gene expression and CRC clinicopathological features, including age (A), gender (B), histological subtype (C), individual cancer stages (D), nodal metastasis status (E), promoter methylation (F), and TP53-mutant (G) in TCGA. (H) The expression of IGF-1R protein in Dukes' A, B, C, and D of CRC (400×, scale = 10 μm). IGF-1R, insulin-like growth factor 1 receptor; CRC, colorectal cancer; TCGA, The Cancer Genome Atlas. *P < 0.05, **P < 0.01, and ***P < 0.001.

Figure 4

Elevated Livin expression is associated with the tumorigenesis of CRC. The correlation between *Livin* gene expression and CRC clinicopathological features, including age (A), gender (B), histological subtype (C), individual cancer stages (D), nodal metastasis status (E), promoter methylation (F), and TP53-mutant (G) in TCGA. (H) The expression of Livin protein in Dukes' A, B, C, and D of CRC (400×, scale = 10 μm). CRC, colorectal cancer; TCGA, The Cancer Genome Atlas. **P < 0.01, ***P < 0.001, and N.S. no significance.

Figure 5

Elevated IGF-1R and Livin expression are not associated with poor prognosis in CRC patients.

(A and B) Survival map analyses of *IGF-1R* and *Livin* gene expression in TCGA. (C and D) Kaplan-Meier survival curves of OS and DFS for CRC patients between high- and low-IGF-1R expression groups in TCGA. (E and F) Kaplan-Meier survival curves of OS and DFS for patients with CRC between high and low Livin expression groups in TCGA. The blue curve represents the low IGF-1R and Livin expression groups. The red curve represents the high IGF-1R and Livin expression group. IGF-1R, insulin-like growth factor 1 receptor; TCGA, The Cancer Genome Atlas; OS, overall survival; DFS, disease-free survival; CRC, colorectal cancer.

Figure 6

Correlation between IGF-IR and Livin expression in CRC. The correlation between IGF-1R and Livin expression in TCGA-COAD by Spearman's chi-square test analysis. The non-log scale for calculation and the log-scale axis for visualization. TCGA, The Cancer Genome Atlas; COAD, colorectal adenocarcinoma.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx