

AVL9 Promotes Colorectal Carcinoma Cell Migration via Regulating EGFR Expression

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Research

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Abstract

Background: Despite advanced treatments could inhibit progression of colorectal carcinoma (CRC), the recurrence and metastasis remain challenging issues. Accumulating evidences implicated that AVL9 played a vital role in human cancers, but it's biological function and mechanism in CRC remain unclear.

Aim: To investigate the biological role and mechanism of AVL9 in colorectal carcinoma.

Results: AVL9 expression was significantly upregulated in tumor tissues than that in matched normal tissues both at mRNA and protein levels. High expression of AVL9 was closely correlated with M status, stages and poor prognosis of colorectal carcinoma (CRC) patients. Functionally, AVL9 overexpression promoted cell migration rather than cell proliferation in vitro, whereas AVL9 knockdown exhibited the contrary results. Mechanistically, AVL9 regulated EGFR expression, and knockdown of EGFR restrained AVL9-induced cell migration.

Conclusion: These findings demonstrated that AVL9 contributed to CRC cell migration by regulating EGFR expression, implying a potential biomarker for CRC early diagnosis.

Background

Colorectal carcinoma (CRC), ranking as one of the most common cancers among males and females, is an important threat to human health [1]. The incidence of colorectal cancer is second only to lung cancer and breast cancer [2]. CRC is a multifactorial disease that can be caused by risk factors such as age, obesity, smoking habit, alcohol use and family history [3]. Intrusive procedures, such as colonoscopy, are the primary diagnostic means [4]. With progression of the disease, non-visible micro-metastases may occur while no obvious symptoms in the early stage [5]. Clinical limitations, specifically, resection evasion during surgery and insufficiently treatment with chemotherapy, lead to a low 5-year survival rate [6, 7]. Therefore, it's urgent to provide a novel biomarker for CRC patients.

AVL9, an exocytosis gene in yeast [8], was first defined to be involved in cell polarity and cell migration [9]. Notably, previous studies suggested that AVL9 was crucially important to tumorigenesis. For instance, Liang et al reported that AVL9 may be served as a direct target of miR-203a-3p in non-small-cell lung cancer (NSCLC). The functional assays indicated that overexpressed miR-203a-3p suppressed cell proliferation, migration and invasion by targeting AVL9 in NSCLC cells [10]. In addition, CRPAT4, hypoxia-regulated lncRNA, worked as an oncogene in cell proliferation and migration via facilitating AVL9 translation in clear cell renal cell carcinomas (ccRCC) [11]. What's more, AVL9 was confirmed as a direct target of miR-497-5p in CRC. And mechanistic studies showed that downregulated Linc00662 significantly suppressed CRC progression and metastasis attributed to compete with miR-497-5p to modulate the expression of AVL9 [12]. Nevertheless, the specific role of AVL9 in CRC is still unknown.

In current study, data from the cancer genome atlas (TCGA) database showed that expression of AVL9 was observably increased in cancer tissue compared to non-cancer tissue, as was consistent with our

results of IHC analysis. Interestingly, biological experiments demonstrated that AVL9 played a vital effect on CRC cell metastasis, but not on proliferation. Moreover, mechanistic researches revealed that AVL9 promoted CRC cell migration via regulating EGFR expression, implying that AVL9 has the potential to be a biomarker for early diagnosis and a novel therapeutic target.

Results

AVL9 is upregulated in colorectal carcinoma

To investigate the role of AVL9 in CRC, we analyzed AVL9 expression at mRNA level in colorectal carcinoma based on TCGA database. The results showed that AVL9 expression was substantially higher in cancer tissues (n=286) than that in normal tissues (n=41) (Fig. 1A). Meanwhile, in accordance with clinical data from TCGA database, expression of AVL9 increased gradually from earlier stage to advanced stage of CRC (Fig. 1B). Next, in order to detect AVL9 expression at protein level, we analyzed the tissue microarray containing 83 tumor samples and 83 non-tumor tissues. Consistent with the above bioinformatics, increased protein level of AVL9 expression in CRC tumor tissues than that in the paired normal tissues was observed by IHC staining. AVL9 expression was upregulated 50/83 (60.2%) in CRC samples (n=83) on the basis of classification of staining results as mentioned before (Fig. 1C). Among them, advanced stages (III + IV) showed strong staining, however, earlier stage (I + II) showed weak staining, indicating that AVL9 expression was positive correlation with stage of CRC (Fig. 1D, $P < 0.05$). Similarly, higher expression of AVL9 was distinctly related to M status ($P = 0.001$) and stages ($P = 0.048$), rather than other clinicopathological characteristic, such as gender, age, tumor size, differentiation, T classification and N status (Table 1). Moreover, compared to lower AVL9 expression at earlier stages (I + II), patients with higher AVL9 expression at advanced stages (III + IV) showed a significantly lower overall survival rate (Fig. 1E). Collectively, data showed above reveals that AVL9 is upregulated in CRC samples and high expression was associated with CRC progression.

AVL9 enhances the CRC cell migration in vitro

In order to further explore biological function of AVL9 in CRC progression, we surveyed AVL9 expression at protein level in five kinds of CRC cell lines by western blot assay (Fig. 1F). Three kinds of CRC cell lines (DLD1, HCT116 and RKO) were selected for subsequent studies. DLD1 and HCT116, low-expressed AVL9, were overexpressed by transiently transfecting with AVL9 overexpressed plasmid. Transfection efficiency was validated by western blot assay (Fig. 2A). The proliferation assay of DLD1 cells transfected with AVL9 plasmid showed no significantly change of growth rates than the control group, which is consistent in HCT116 cells (Fig. 2B). To examine the effect of AVL9 overexpression on the cell migratory ability, DLD1 cell stably overexpressing AVL9 were established and then trans-well assay was carried out. The results indicated that AVL9 could markedly increase the DLD1 cell migration (Fig. 2C). Subsequently, scratch-wound healing assay was performed. Cells overexpressed AVL9 showed stronger wound healing ability compared to control group, suggesting a positive impact of AVL9 on CRC cell migration (Fig. 2D). These findings collectively implicate that AVL9 facilitates the CRC cell migration in vitro.

Knockdown of AVL9 significantly inhibits CRC cell migration in vitro

Next, loss-of-function experiments were carried out to explore the role of AVL9 in RKO cell line. Two AVL9-specific siRNA1, siRNA2 and NC siRNA were constructed and respectively transfected in the RKO cell. Transfection efficacy was confirmed by western blot analysis (Fig. 3A). Cell counting for five consecutive days (CCK-8 assay) was performed to assess the potential effects upon downregulating AVL9 expression on cell proliferation. As is shown in Fig. 3B, no remarkable changes were observed. Trans-well assay demonstrated that stably silencing AVL9 in RKO cell significantly inhibited the cell migration compared with the control group (Fig. 3C). In addition, wound healing assay, measurements of cell motility after 24h, revealed that AVL9 knockdown could restrained the migration of RKO cell (Fig. 3D). These data further confirm that AVL9 performs a crucial role in CRC cell migration, rather than cell proliferation.

AVL9 regulates EGFR expression in CRC cells

To investigate the molecular mechanism of how AVL9 promotes CRC cells migration, western blot analysis was performed to examine the expression of tumor-related proteins, including E-cadherin, EGFR, FAK, PTEN, P53, CDK4 and CDK6. As is shown in Fig. 4A, EGFR expression at protein level decreased following AVL9 knockdown in RKO cell. Consistent and significant conclusion were obtained through AVL9 overexpression in HCT116 cell (Fig. 4B). Epidermal Growth Factor Receptor (EGFR), correlated positively with cancers progression and poor prognosis, was reported highly expressed in different types of tumors [13, 14]. Thus, we supposed that AVL9 promotes cell migration mainly via regulating EGFR expression in CRC. For patients with metastatic CRC (mCRC), cetuximab (CTX), as an EGFR monoclonal antibody, is a typical anti-cancer therapeutic method to suppress cancer progression binding with traditional chemotherapy [15, 16]. To further validate the above assumption, we suppressed EGFR expression in AVL9 overexpressed cell by cetuximab treatment (1 μ M) and performed the rescue experiments. Trans-well assay revealed that decreased EGFR expression could partially inhibit the increased migration caused by AVL9 overexpression in DLD1 cell, indicating that AVL9 promotes CRC cells migration via regulating EGFR expression (Fig. 4C). EGFR, a well-described transmembrane protein, is a member of ERBB receptor tyrosine kinase superfamily which promotes tumor cell migration [17]. Subsequently, GSEA (gene set enrichment analysis) was performed to drive enrichment scores between high expression of AVL9 tumor group and low expression of AVL9 tumor group based on TCGA datasets. Notably, the result revealed that ERBB signaling pathway was positively associated with AVL9 expression based on the CRC GEO datasets (Fig. 4D). These collective findings support that EGFR/ERBB signaling pathway may be essential for AVL9 mediated migration in CRC.

Discussion

Incidence and mortality of CRC have been showed an increasing trend in China according to the global estimates for cancers worldwide report. Searching for the robust molecular markers is a common focus in facilitating the diagnosis and prognosis of colorectal carcinoma disease [18]. Utility of several genes has been explored that in CRC [19]. AVL9, a novel protein associated with establishing and maintaining

epithelial cell polarity, is identified as an exocytosis gene in yeast [9, 10]. It has been reported that the loss of cell polarity acted as a hallmark of epithelial cancers, indicating that epithelial polarity is involved in cancer tumorigenicity and migration [20–26]. To determine whether AVL9 functions in cancer, Zhang et al reported that CRPAT4, hypoxia-regulated lncRNA, promotes clear cell renal cell carcinomas cell migration via modulating AVL9 [11]. In addition, in non-small-cell lung cancer, AVL9 was found could be functioned as a direct target of miR-203a-3p [10]. Accumulating studies confirmed that AVL9 could be a downstream gene involved in the tumor proliferation and migration. However, the specific role and biological function about AVL9 alone in CRC has not been elucidated.

In this study, we analyzed AVL9 mRNA expression in colorectal carcinoma based on TCGA database. Results revealed that AVL9 mRNA expression was significantly increased in CRC tissue compared to corresponding non-cancer tissue. Meanwhile, expression of AVL9 at mRNA level increased gradually from earlier stage to advanced stage of CRC. Consistent with the above bioinformatics, increased protein level of AVL9 expression in CRC tumor tissues than that in the paired normal tissues was observed by IHC staining and AVL9 expression at protein level was positive correlation with stage of CRC. Moreover, we found that higher expression of AVL9 was relevant to M status and stages, unfavorable to prognosis in CRC patients, and there is no relationship between AVL9 and other clinicopathological variables such as gender, age, tumor size, differentiation, T classification and N status. To further explore the potential action of AVL9, CCK-8 assay and trans-well assay were conducted respectively. No obvious difference was found on the CRC cell proliferation upon interfering the expression of AVL9, but interestingly, cell migration was significantly influenced, strongly supporting the notion that AVL9 is migration-associated gene. Mechanically, we found that overexpressed AVL9 increased EGFR protein expression while downregulated AVL9 suppressed EGFR protein expression. Epidermal growth factor receptor (EGFR), a 170-kDa transmembrane glycoprotein composed of an intracellular tyrosine-kinase domain, is one of the anticancer drug targets for colorectal cancer [27]. Recently, EGFR has been reported to be elevated and correlated with advanced tumor stage and increased risk of metastasis in CRC [28–30]. Rescue experiment showed that cetuximab, an EGFR monoclonal antibody, attenuated the effect of upregulated AVL9 on the CRC cell migration promotion. Considering that EGFR is a member of ERBB receptor tyrosine kinase superfamily which promotes tumor cell migration. GSEA analysis suggested that ERBB signaling pathway was positively associated with AVL9 in CRC. These results indicated that EGFR serves as the downstream effector of AVL9 in modulating CRC cell migration. Given this, further investigation is required for a better understanding about the underlying mechanism.

Taken together, we demonstrated that AVL9 was upregulated in CRC and it could be functioned as an oncogene in CRC progression, facilitating cell migration via regulating EGFR expression, rather than cell proliferation. Despite further studies are needed to be explored for precise interaction between AVL9 and EGFR/ERBB signaling pathway in CRC, findings we found provided a novel insight into the potential biomarker for CRC diagnosis and the risk stratification factor for CRC patients.

Materials And Methods

Tissue microarray and immunohistochemistry staining

The tissue microarray construction (TMA, #HStmA150CS02) containing 83 human CRC cancer specimens and 83 non-cancer specimens was bought from Shanghai Outdo Biotech, Shanghai, China and analyzed by two pathologists in a blinded manner. Detailed clinical information is included, for example, gender, age, tumor size, differentiation, T classification, N status, M status and stages. An anti-AVL9 antibody (ab175108; Abcam, Cambridge, MA, USA) at a dilution of 1:500 was used to detect the expression of AVL9. The classification of ICH staining (immunohistochemical staining) results was as mentioned [31]. The study was approved by the ethics committee of Shanghai East Hospital, Tongji University School of Medicine.

Cell lines and culture condition

Five kinds of human CRC lines (HCT116, DLD1, RKO, LOVO, SW620) obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China), were cultured in Dulbecco's modified Eagle's medium (DMEM; Corning, Inc, Corning, NY, USA) supplemented with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin (M&C Gene Technology Ltd, Beijing, China). All the cells were incubated in a humidified environment at 37°C with 5% CO₂.

siRNA interference, plasmid construction and transfection.

The expression of AVL9 in CRC cells were downregulated by transient with two different siRNAs which were synthesized by GenePharma, Shanghai, China. The sense sequences of siRNA were designed as follow: siAVL9-1:5-GCCACAGUAAUUGGUAUCUdTdT-3, siAVL9-2:5-GGACUCUUCAUGGCAUCAAdTdT-3, siNC:5-UUCUCCGAACGUGUCACGUDTdT-3. Overexpressed plasmid, a full-length AVL9 cDNA was cloned into pcDNA 3.1 mammalian expression vector, was purchased from Vigene Biosciences, Shandong, China. Following the manufacturer's instructions, siRNAs or plasmids were transfected using lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific, US). In order to stably overexpressed or knockdown the expression of AVL9, Lentiviral particles (Lv6-AVL9 and Lv6-NC; Lv3-shAVL9 and Lv3-shNC based on siAVL9-1 and siNC sequence) was purchased from Gene Pharma, Shanghai, China. CRC cells stably infected cells were selected by puromycin (2mg/ml) after transducing cells with lentiviral particles.

CCK-8 assay

Proliferation assay, the cell counting kit-8(CCK-8) assay, was conducted in accordance with the manufacturer's instructions. In brief, the appropriate density of CRC cells transfected with siRNA or plasmid were seeded into 96-well plate per cell culture condition above mentioned. Each well was treated with 10ul CCK-8 reagent (Dojindo, Japan) and continued to be cultured for 1.5h. Then, the absorbance value at 450nm was measured spectrophotometrically for five consecutive days. Each experiment was independently performed 3 times in triplicate.

Cell migration assay

Trans-well assay was performed to measure cell migration using a 24-well trans-well chambers (pore size, 8µm; Costar; Corning, Inc). 5×10^4 cells counted were cultivated in the upper chamber containing 400µl DMEM free FBS while 800µl DMEM with 10% FBS were supplemented in the bottom chambers. After 24-28h incubation, migrated cells at the bottom of chamber were stained with 0.5% crystal violet and calculated with microscope. Three independent assays with triplicate was performed.

Wound healing assay

CRC cells stably overexpressing or knocking down AVL9 and corresponding control cells seeded in 6-well plate at 37°C with 5% CO₂ were scratched by a sterile 200µL plastic tip (90% confluence). Firstly, rinse off the dead cells with 1x PBS. Then, the cells were cultivated in serum-free DMEM medium for up to 48 h culture. Pictures were taken by the light microscope (Nikon, Japan) with a microscope at 200× magnification. The experiments were performed at least three times.

Western blot assay

Western blot analysis was carried out as described previously [32]. The Primary antibodies used in this study are mainly: anti-AVL9 (ab175108, Abcam, 1:500), anti-E-cadherin (#3195, Cell Signaling Technology, 1:1000), anti-β-actin (sc-58673, Santa Cruz Biotechnology, 1:500), anti-p53 (#sc-47698, Santa Cruz Biotechnology, 1:500), anti-PTEN (#559600, BD Biosciences, 1:500), anti-EGFR (#4267, Cell Signaling Technology, 1:500), anti-CDK4(#12790, Cell Signaling Technology, 1:800), anti-CDK6 (#14052-1-AP, ProteinTech,1:500), anti-FAK (#66258-1-Ig, Proteintech, 1:500). Secondary antibodies are as follows: anti-rabbit-DyLight 800 (#SA5–35571, 1:1000, Thermo Fisher Scientific) and anti-mouse-DyLight 800 (#SA5–35521, 1:1000, Thermo Fisher Scientific).

Datasets and Gene set enrichment analysis (GSEA)

The public data, gene expression at mRNA levels in tumor and corresponding non-tumor CRC tissue and, were obtained using GEPIA online tools based on TCGA database (<http://gepia.cancer-pku.cn/>). GSEA analysis was performed to explore biological signaling pathway between the AVL9 high expression group and the AVL9 low expression group. In detail, GSEA analysis was applied using GSEA 4.0.3 (<https://www.gsea-msigdb.org/gsea/index.jsp>).

Data accessibility

Gene analysis in TCGA dataset was performed by online tool GEPIA (<http://gepia.cancer-pku.cn/>).

Statistical analysis

Statistical analysis for three independent experiments was analyzed by GraphPad Prism 8. The quantitative data was represented as mean ±SD. Difference comparison were analyzed by Student *t* test or one-way ANOVA. Relationships between AVL9 expression and clinicopathological characteristics were analyzed using χ^2 test. $P < 0.05$ was served to show statistical significance.

Abbreviations

CRC: colorectal carcinoma; NSCLC: non-small-cell lung cancer; ccRCC: clear cell renal cell carcinomas; TCGA: the cancer genome atlas; EGFR: epidermal growth factor receptor; FAK: focal adhesion kinase; PTEN: phosphatase and tensin homolog; CDK4: cyclin dependent kinase 4; CDK6: cyclin dependent kinase 6; mCRC: metastatic colorectal carcinoma; CTX: cetuximab; GSEA: gene set enrichment analysis; TMA: tissue microarray construction; IHC staining: immunohistochemical staining.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data analyzed and generated in this study are included in this published article.

Competing interests

The authors declare no potential conflict of Interest.

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Authors' contributions

JingDe Chen and Zhuqing Zhou designed the research; Qiong Wu performed the research and analyzed the data. Qiong Wu and JingDe Chen wrote the paper. All authors read and approved the final manuscript.

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Not applicable.

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References

1. S.H. Wong, J. Yu, Gut microbiota in colorectal cancer: mechanisms of action and clinical applications, *Nat Rev Gastroenterol Hepatol*, 2019;16:690-704.
2. J. Ferlay, M. Colombet, I. Soerjomataram, et al., Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, *Int J Cancer*, 2019;144:1941-1953.
3. V. Aran, A.P. Victorino, L.C. Thuler, et al., Colorectal Cancer: Epidemiology, Disease Mechanisms and Interventions to Reduce Onset and Mortality, *Clin Colorectal Cancer*, 2016;15:195-203.
4. M. Kurt, Z. Yumuk, Diagnostic accuracy of *Fusobacterium nucleatum* IgA and IgG ELISA test in colorectal cancer, *Sci Rep*, 2021;11:1608.
5. W.Q. Liu, W.L. Li, S.M. Ma, et al., Discovery of core gene families associated with liver metastasis in colorectal cancer and regulatory roles in tumor cell immune infiltration, *Transl Oncol*, 2021;14:101011.
6. C. Hasovits, S. Clarke, Pharmacokinetics and pharmacodynamics of intraperitoneal cancer chemotherapeutics, *Clin Pharmacokinet*, 2012;51:203-224.
7. V. Heinemann, L.F. von Weikersthal, T. Decker, et al., FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial, *Lancet Oncol*, 2014;15:1065-1075.
8. E. Harsay, R. Schekman, Avl9p, a member of a novel protein superfamily, functions in the late secretory pathway, *Mol Biol Cell*, 2007;18:1203-1219.
9. J. Tang, Y. Li, K. Lyon, et al., Cancer driver-passenger distinction via sporadic human and dog cancer comparison: a proof-of-principle study with colorectal cancer, *Oncogene*, 2014;33:814-822.
10. J. Liang, T. Sun, G. Wang, et al., Clinical significance and functions of miR-203a-3p/AVL9 axis in human non-small-cell lung cancer, *Per Med*, 2020;17:271-282.
11. W. Zhang, J. Wang, R. Chai, et al., Hypoxia-regulated lncRNA CRPAT4 promotes cell migration via regulating AVL9 in clear cell renal cell carcinomas, *Onco Targets Ther*, 2018;11:4537-4545.
12. H. Wang, M. Yu, W. Hu, et al., Linc00662 Promotes Tumorigenesis and Progression by Regulating miR-497-5p/AVL9 Axis in Colorectal Cancer, *Front Genet*, 2019;10:1385.
13. P. Seshacharyulu, M.P. Ponnusamy, D. Haridas, et al., Targeting the EGFR signaling pathway in cancer therapy, *Expert Opin Ther Targets*, 2012;16:15-31.
14. M. Jozkowiak, M. Dyszkiewicz-Konwinska, P. Ramlau, et al., Individual and Combined Treatments with Methylated Resveratrol Analogue DMU-214 and Gefitinib Inhibit Tongue Cancer Cells Growth via Apoptosis Induction and EGFR Inhibition, *Int J Mol Sci*, 2021;22.
15. C. Jing, R. Ma, H. Cao, et al., Long noncoding RNA and mRNA profiling in cetuximab-resistant colorectal cancer cells by RNA sequencing analysis, *Cancer Med*, 2019;8:1641-1651.
16. A.P. Venook, D. Niedzwiecki, H.J. Lenz, et al., Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial, *JAMA*, 2017;317:2392-2401.

17. A. Appert-Collin, P. Hubert, G. Cremel, et al., Role of ErbB Receptors in Cancer Cell Migration and Invasion, *Front Pharmacol*, 2015:6:283.
18. F. Bray, J. Ferlay, I. Soerjomataram, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J Clin*, 2018:68:394-424.
19. C. Royer, X. Lu, Epithelial cell polarity: a major gatekeeper against cancer?, *Cell Death Differ*, 2011:18:1470-1477.
20. T. Igaki, R.A. Pagliarini, T. Xu, Loss of cell polarity drives tumor growth and invasion through JNK activation in *Drosophila*, *Curr Biol*, 2006:16:1139-1146.
21. A. Wodarz, I. Nathke, Cell polarity in development and cancer, *Nat Cell Biol*, 2007:9:1016-1024.
22. M. Lee, V. Vasioukhin, Cell polarity and cancer—cell and tissue polarity as a non-canonical tumor suppressor, *J Cell Sci*, 2008:121:1141-1150.
23. M.J. Bissell, W.C. Hines, Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression, *Nat Med*, 2011:17:320-329.
24. A. Linford, S. Yoshimura, R. Nunes Bastos, et al., Rab14 and its exchange factor FAM116 link endocytic recycling and adherens junction stability in migrating cells, *Dev Cell*, 2012:22:952-966.
25. M. Krishnan, L.A. Lapierre, B.C. Knowles, et al., Rab25 regulates integrin expression in polarized colonic epithelial cells, *Mol Biol Cell*, 2013:24:818-831.
26. M.E. Feigin, S.D. Akshinthala, K. Araki, et al., Mislocalization of the cell polarity protein scribble promotes mammary tumorigenesis and is associated with basal breast cancer, *Cancer Res*, 2014:74:3180-3194.
27. L. Repetto, W. Gianni, A.M. Agliano, et al., Impact of EGFR expression on colorectal cancer patient prognosis and survival: a response, *Ann Oncol*, 2005:16:1557.
28. R. Radinsky, Modulation of tumor cell gene expression and phenotype by the organ-specific metastatic environment, *Cancer Metastasis Rev*, 1995:14:323-338.
29. R. Radinsky, S. Risin, D. Fan, et al., Level and function of epidermal growth factor receptor predict the metastatic potential of human colon carcinoma cells, *Clin Cancer Res*, 1995:1:19-31.
30. N.S. Goldstein, M. Armin, Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: implications for a standardized scoring system, *Cancer*, 2001:92:1331-1346.
31. N. Dou, D. Yang, S. Yu, et al., SNRPA enhances tumour cell growth in gastric cancer through modulating NGF expression, *Cell Prolif*, 2018:51:e12484.
32. S. Yu, L. Li, H. Cai, et al., Overexpression of NELFE contributes to gastric cancer progression via Wnt/beta-catenin signaling-mediated activation of CSNK2B expression, *J Exp Clin Cancer Res*, 2021:40:54.

Tables

Table 1 The correlation of AVL9 expression with clinicopathological features of colorectal carcinoma patients

	Number of cases (n=83)	Negative, weak (n=50)	Moderate, strong (n=33)	<i>P</i> value
Age (years)				
> 50	60	33	27	0.115
≤ 50	23	17	6	
Gender				
Male	50	27	23	0.152
Female	33	23	10	
Tumor size				
≤ 5 cm	50	28	22	0.331
> 5 cm	33	22	11	
T classification				
T1,T2	17	12	5	0.328
T3,T4	66	38	28	
N status				
N0	52	29	23	0.281
N1-3	31	21	10	
Metastasis				
M0	35	14	21	0.001
M1	48	36	12	
Stage				
I,II	32	15	17	0.048
III, IV	51	35	16	

Figures

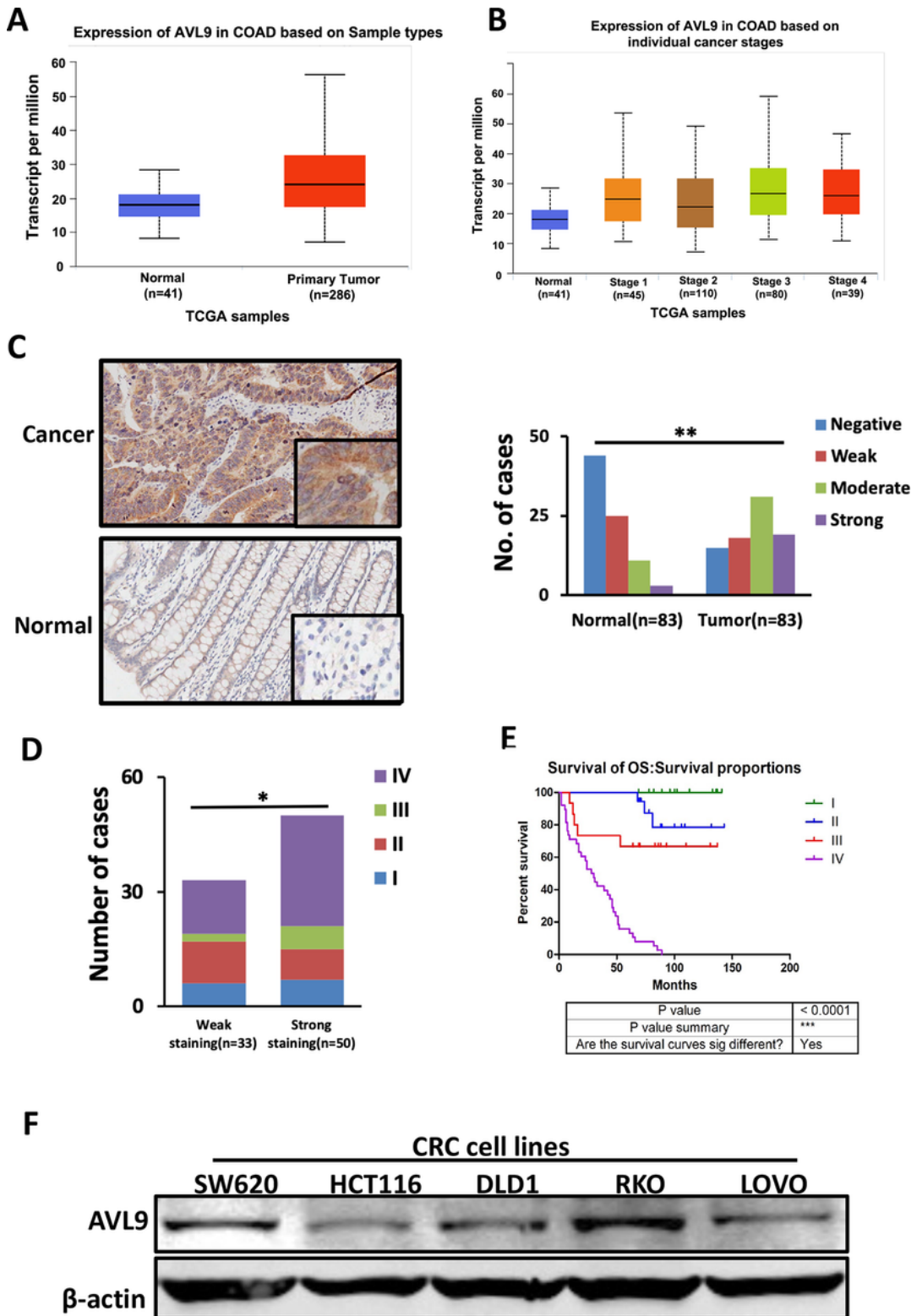


Figure 1

AVL9 was aberrantly elevated in colorectal carcinoma. (A) The expression of AVL9 at mRNA level in CRC tissue was obtained from TCGA database. (B) AVL9 expression was positive correlation with stages of CRC tissue. (C) Representative pictures of CRC samples with IHC staining was shown. (D) Statistical analysis of AVL9 staining strength in a tissue microarray containing 83 pairs of CRC samples. Chi-square test was used. (E). Overall survival rate was shown at different stages of CRC patients. (F) Relative AVL9

expression at protein level in CRC cell lines was determined by western blot analysis. *p < 0.05, **P<0.01, ***P<0.001.

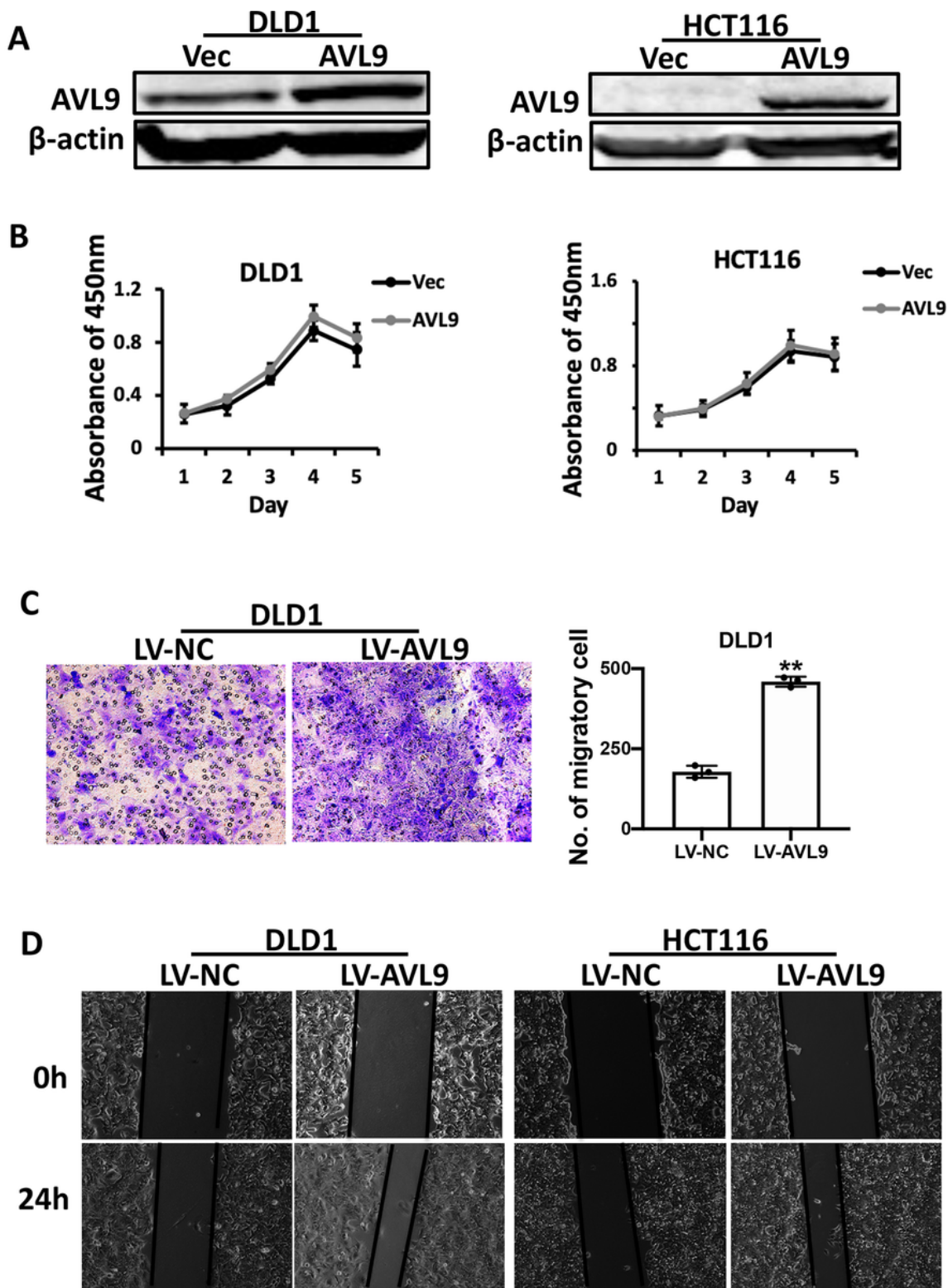


Figure 2

Effects of AVL9 overexpression on CRC cells proliferation and migration in vitro. Notes: DLD1 and HCT116 cells were transfected with plasmids for 24h respectively. (A) The overexpression efficacy of AVL9 was verified by western blot assay. (B) CCK-8 assay showed that no remarked change was

observed following AVL9 overexpression in DLD1 and HCT116 cells; however, AVL9 overexpression significantly promoted cell migration which were determined by Trans-well assay(C) and Wound healing assay(D). All the experiments were performed at least three times. All values were represented as means \pm SEM. * $p < 0.05$.

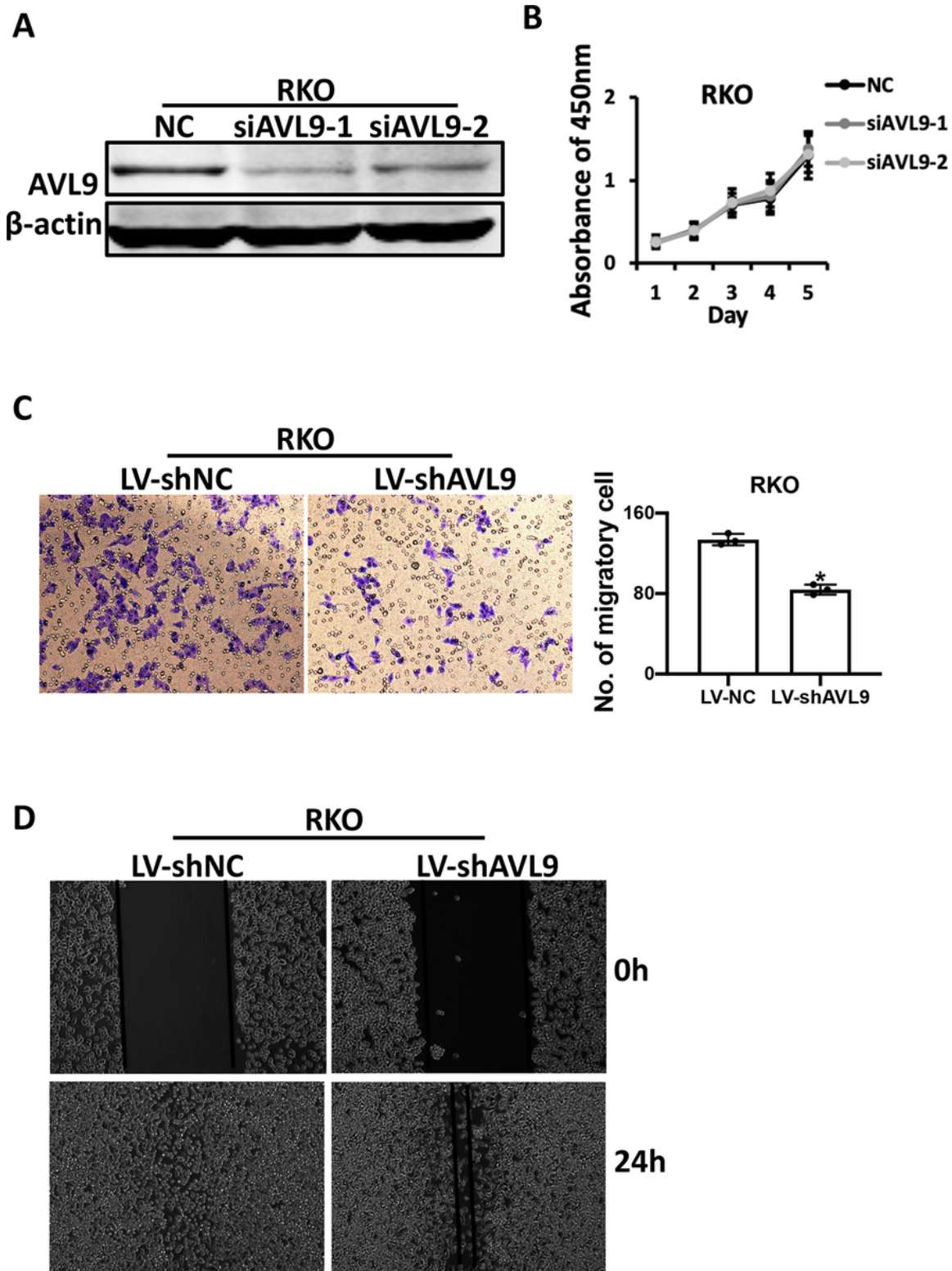


Figure 3

AVL9 knockdown inhibited RKO cell migration. AVL9 expression was downregulated in RKO cell by transfecting specific siRNA (siAVL9-1/siAVL9-2) which was confirmed by western blot assay(A). Effect of AVL9 knockdown on cell growth was detected by CCK-8 assay(B). AVL9 silencing decreased the migration of RKO cell verified by Trans-well assay(C) and Wound healing assay(D). Data was represented as means \pm SEM. of three replicates. * $p < 0.05$.

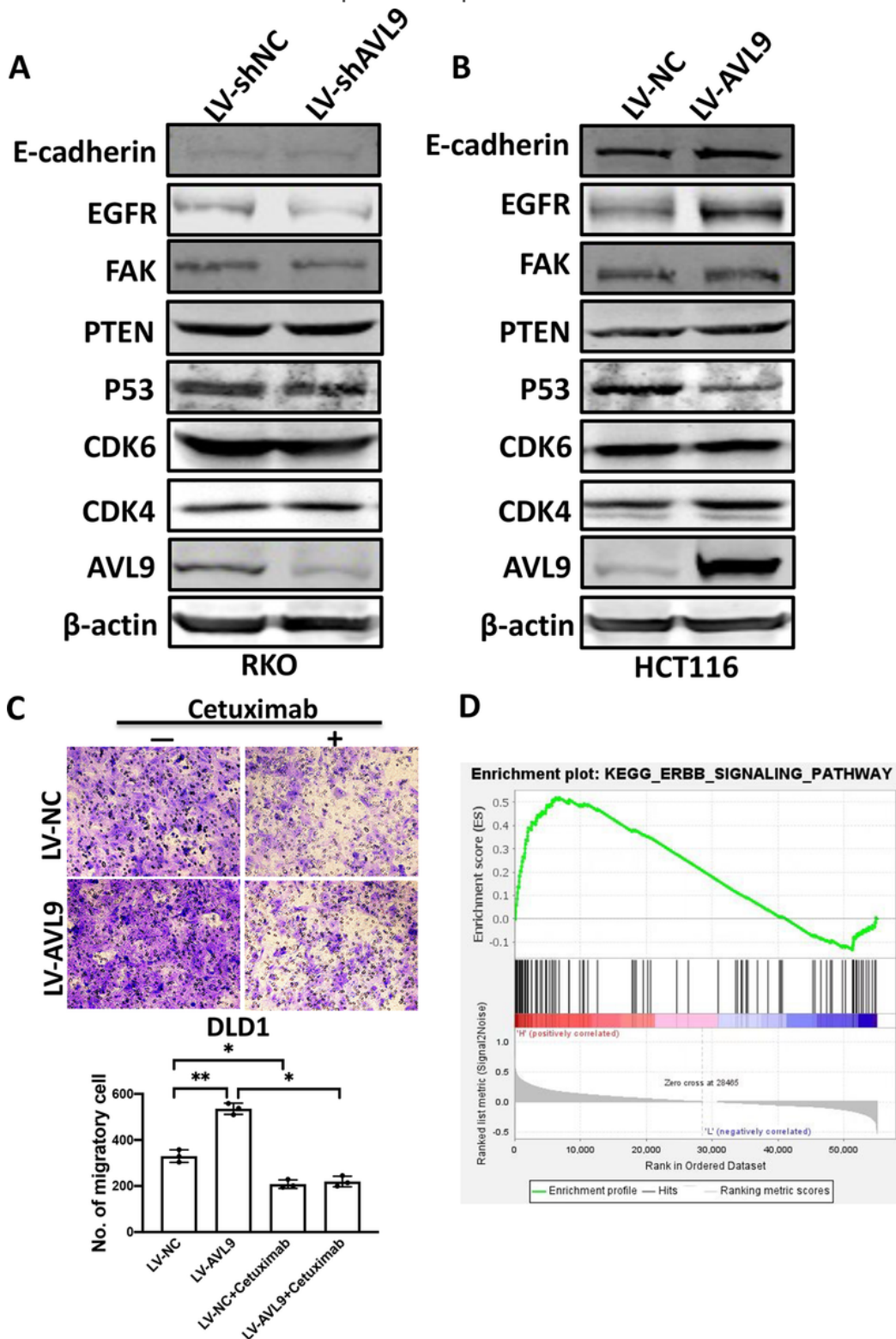


Figure 4

AVL9 upregulated EGFR protein level expression. (A) Western blot analysis confirmed the effect of AVL9 on tumor-related factors in RKO-Lv3-shAVL9/ RKO-Lv3-shNC and HCT116-Lv6-AVL9/HCT116-Lv6-NC cells. Silencing AVL9 reduced the expression of EGFR at protein level in RKO-Lv3-shAVL9 cell compared to RKO-Lv3-shNC, simultaneously, AVL9 overexpression upregulated the protein level of EGFR expression in HCT116-Lv6-AVL9 compared to the control group (B). (C) Rescue experiment showed that decreased EGFR expression could partially suppressed the increased migration in DLD1-Lv6-AVL9 compared to DLD1-Lv6-NC cell. Cells were treated or not with concentrations of cetuximab (1uM). (D) GSEA showed that ERBB signaling pathway was upregulated in AVL9 high expression group. The CRC patients were categorized into high expression group and low expression group by mean expression of AVL9. *p < 0.05, **P<0.01.