

DPEP1 Increases Drug Resistance in an ASCL2-Dependent Manner in Colon Cancer Cells and Correlates with Immune Cell Infiltration

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Research Article

Keywords: DPEP1, ASCL2, colon cancer, immune infiltration, drug resistance, bioinformatics

Posted Date: November 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1022827/v1>

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Abstract

Background: Dipeptidase 1 (DPEP1) is associated with several human cancers. However, its function in colon cancer remains unclear.

Methods: DPEP1 expression was analyzed by TCGA and GTEx pancancer data. Survival analysis was performed using the R survival package to assess the prognostic value of the DPEP1 expression level in colon cancer. DPEP1 enrichment analysis was conducted by the *clusterProfiler* R software package. Correlation analysis, differential expression analysis, and Venn analysis were used to obtain key genes. Correlation of DPEP1 or ASCL2 expression with immune cell infiltration in colon cancer was performed by TIMER and GSCA databases. qRT-PCR, Western blot, Co-immunoprecipitation, dual luciferase reporter experiments, and immunohistochemistry were used to explore the correlation between DPEP1 and ASCL2. MTT was used to evaluate the role of DPEP1 in colon cancer drug resistance.

Results: DPEP1 was overexpressed in various cancers, including colon cancer. High DPEP1 expression was negatively correlated with the disease-specific survival (DSS) and progression-free interval (PFI) but not significantly correlated with the overall survival (OS). Enrichment analysis showed that DPEP1 may be related to the Wnt signaling pathway and Hippo signaling pathway. Further analysis showed that DPEP1 and ASCL2 had a strong positive correlation, and both correlated with immune cell infiltration. Moreover, DPEP1 enhanced drug resistance in an ASCL2-dependent manner.

Conclusions: Our findings revealed that DPEP1 enhanced drug resistance in an ASCL2-dependent manner and correlated with immune infiltration in colon cancer.

Introduction

Colorectal cancer is a common malignant tumor in the digestive system. Its global incidence rate is lower than that of breast cancer and lung cancer, as it ranks third, and its mortality rate ranks second in cancer-related deaths[1]. The occurrence of colon cancer is currently believed to be related to genetics, environment, diet, inflammatory bowel disease, sex, race, etc.[2]. However, the exact molecular mechanism of its pathogenesis is still unclear. With the rapid development of high-throughput sequencing and gene chip technology, an increasing number of driver genes have been discovered[3]. However, discovering additional important driver oncogenes, especially those that affect immune cell infiltration in the tumor microenvironment and increase resistance to chemotherapeutic drugs in colon cancer, is very important.

Dipeptidase 1 (DPEP1) is a zinc-dependent metalloproteinase that participates in antibiotic processing, dipeptide hydrolysis, and glutathione and leukotriene metabolism[4, 5]. Recently, more attention has been given to the effect of DPEP1 on cancers. Studies have shown that DPEP1 can promote hepatoblastoma progression and leukemia cell proliferation[6, 7]. In colorectal cancer, DPEP1 expression is upregulated, and the DPEP1 high-expression group has a poor prognosis[8], and DPEP1 is considered a specific tumor marker for colon cancer[9]. Moreover, DPEP1 promotes colon cancer cell proliferation, metastasis, and

invasion[10–12]. Recently, single-cell RNA sequencing indicated that DPEP1 plays a key role in the evolution process from ulcerative colitis to ulcerative colitis-associated colon cancer[13]. However, the underlying mechanism of DPEP1 in colon cancer drug resistance and tumor immunology remains elusive.

In our study, we analyzed the colon cancer data in the TCGA database and GEO database through bioinformatics analysis and found that DPEP1 is significantly highly expressed in colon cancer. Moreover, high DPEP1 expression was correlated with poor disease-specific survival (DSS) and progression-free interval (PFI) in colon cancer patients. Subsequently, through bioinformatics analysis, we found that the expression and immune infiltration of ASCL2 have a significant positive correlation with that of DPEP1 in colon cancer samples. Immunohistochemical and Western blot results also showed that the expression of DPEP1 and ASCL2 were positively correlated with each other in colon cancer samples. Interestingly, we found that DPEP1 could upregulate the ASCL2 expression in the protein levels. Mechanism studies found that DPEP1 inhibited the ubiquitination degradation of ASCL2 by maintaining the stability of ASCL2. In turn, ASCL2 functioned as a transcription factor to regulate DPEP1 expression at the transcriptional level. Functionally, DPEP1 expression could increase the resistance of colon cancer cells to chemotherapy drugs oxaliplatin or irinotecan respectively. In this way, DPEP1 and ASCL2 formed a positive feedback loop regulation mode, which increased the tolerance of tumor cells to chemotherapeutic drugs. Thus, our studies offer novel insights into the functional role of DPEP1 in colon cancer.

Materials And Methods

Tissue samples and cell lines

Colon cancer cell lines, including HCT116, SW480, SW620 and RKO, were purchased from the Cell Bank at the Shanghai Institute of Cells, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM or 1640 medium (Gibco, USA) supplemented with 10% FBS and 1% penicillin/streptomycin (Beyotime, China) at 37°C with 5% CO₂. Four pairs of colon cancer and adjacent normal tissues were obtained from Wujin Hospital affiliated with Jiangsu University. Informed consent forms were obtained from patients. This study was approved by the Ethics Committee of the Inner Mongolia People's Hospital.

Data collection and analysis

DPEP1 expression profiles in TCGA and Genotype-Tissue Expression (GTEx) clinical pancancer data were downloaded from the University of California, Santa Cruz (UCSC) Xena database (<https://xenabrowser.net/datapages/>), including data on ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, OV, PAAD, PRAD, READ, SARC, SCKM, STAD, TGCT, THCA, THYM, UCEC, and UCS. To evaluate DPEP1 expression, tumor tissues were obtained from TCGA, and normal tissues were obtained from the TCGA and GTEx databases.

GSE74602, based on the GPL6104 platform, was obtained from the GEO database, including 30 colon cancer tissue samples and 30 paracancerous tissue samples. Differentially expressed genes (DEGs) were

obtained by the R package limma. The false positive results could be corrected by the adjusted P value (adj. P value) of the FDR method. Therefore, the cutoff criteria for DEGs were as follows: adjusted $p < 0.05$ and $|\log_2\text{-fold change (FC)}| > 1.5$.

Survival analysis was performed using the R survival package to assess the prognostic value of the level of DPEP1 expression in colon cancer. Samples were divided into high expression groups and low expression groups with the best cutoff value for DPEP1 mRNA expression in colon cancer, and the OS, DSS and PFI of different expression groups were compared. Survival curves were plotted using the Kaplan-Meier method. Cox regression was used to evaluate statistical significance. $p < 0.05$ was considered statistically significant. Moreover, overall survival analysis for DPEP1 in pan-cancer was conducted.

Correlation and enrichment analysis

Pearson's correlation analysis of DPEP1 mRNA and other mRNAs in colon cancer was performed using TCGA COAD data. The top 200 genes most positively associated with DPEP1 were selected for Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to determine the function of DPEP1. GO enrichment analysis included molecular function, biological process, and cell components. The 'ClusterProfiler' package in R software was used to analyze the functional enrichment with a $p \text{ value} < 0.05$.

Immune cell infiltration analysis

TIMER (<https://cistrome.shinyapps.io/timer/>) is a web tool for the comprehensive analysis of tumor-infiltrating immune cells. We analyzed the correlation of DPEP1 and ASCL2 expression levels with immune cell infiltration levels, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells. In addition, the TIMER database was also used to analyze the correlation of DPEP1 and ASCL2 expression levels with the biomarkers of immune checkpoints in colon cancer. A $p\text{-value} < 0.05$ was considered statistically significant.

GSCA (<http://bioinfo.life.hust.edu.cn/GSCA/#/>) is an integrated database for genomic and immunogenomic gene set cancer analysis and was used to explore the association between immune infiltrate and the expression score compiled by the GSVA of a gene set including DPEP1 and ASCL2.

GEPIA (<http://gepia.cancer-pku.cn/index.html>) is a newly developed interactive web server to analyze the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and GTEx projects, using a standard processing pipeline. GEPIA was utilized to analyze the correlation of DPEP1 and ASCL2 expression levels with biomarkers of immune checkpoints in colon cancer. $|R| > 0.1$ and $p \text{ value} < 0.05$ were considered statistically significant.

Cell transfection

Cell transfection was performed using TurboFect Transfection (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. Briefly, cells were seeded in six-well plates (Corning, USA), grown to a cell

density of 70% and then transfected and cultured at 37°C for 48 h.

RNA extraction and qRT-PCR

Total RNA was isolated using the TRIzol reagent (TaKaRa, JPN) according to the manufacturer's protocol. One milligram of RNA was used for cDNA synthesis using a TaqMan cDNA synthesis kit (TaKaRa, JPN). qRT-PCR was conducted using a SYBR Green PCR Kit (TaKaRa, JPN) on a 7500 Real-time PCR System (TaKaRa, JPN). The primer sequences were listed in **Supplementary Table 1**. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative levels.

Western blotting

The cells were washed with PBS, and then the proteins were extracted using RAPI lysis (Sangon Biotech, China). Proteins were segregated using 8% SDS-PAGE and transferred to PVDF membranes (Millipore, USA). The PVDF membranes were then incubated with DPEP1 (dilution: 1: 1000, Abcam, UK), ASCL2 (dilution: 1: 1000, Abcam, UK), β -actin antibody (dilution: 1: 4000, Sangon Biotech, China) or ubiquitin (dilution: 1: 1000, Boston Biochem, USA) after incubation with goat anti-rabbit or goat anti-mouse IgG HRP antibody (dilution: 1: 5000, Sangon Biotech, China). The bands were measured using an ECL detection kit (Labgic, China). The gray analysis for the protein bands was performed using ImageJ software.

Immunohistochemical staining

Immunohistochemical staining assays were performed according to a published paper[14]. The primary antibody used in the experiments was as follows: ASCL2 (dilution: 1: 100, Abcam, UK) and DPEP1 (dilution: 1: 100, Abcam, UK).

Co-immunoprecipitation (CoIP) assays

Co-immunoprecipitation assays were performed according to previous protocols[14].

Protein stability experiments

HCT116 cells were transiently transfected with pCMV or pCMV-DPEP1 in 6-well plates. SW620 cells were transiently transfected with siRNA (100 nM) or siRNA-DPEP1 (100 nM). After 48 h, the samples were treated with cycloheximide (CHX, Sigma, USA) at a final concentration of 15 mg/ml for 0, 0.5 and 1.0 h. The detail procedures were followed the previous methods[10].

Protein degradation assays

HCT116 cells were transiently transfected with pCMV-DPEP1 or pCMV plasmids. After 48 h incubation, the cells were lysed on ice for 30 min and then centrifuged at 15,000 rpm for 20 min. Lysates were incubated with ASCL2 primary antibody for 4 h at 4°C, and then protein A/G (Invitrogen, USA) was added overnight. Western blotting was performed, and an *anti*-ubiquitin antibody was employed to test the relative degradation levels of ASCL2 proteins in the cells.

Dual luciferase reporter experiments

To test the transcription activity of DPEP1 promoter, we constructed the DPEP1 promoter (-1500bp/+200bp region) into pGL3-Basic plasmids, and then tested the activity of pGL3-DPEP1 promoter by the Dual luciferase reporter experiments. The dual luciferase reporter experiments were performed as previously described[15].

MTT assays

Cells from each group were seeded in 96-well plates at a density of 5000 cells per well and were incubated at 37°C for overnight. The culture medium was replaced with fresh culture medium with different concentrations of oxaliplatin (0, 40 and 80 μ M) or irinotecan (0, 5 and 10 μ M) with 5 replicates each. After 48 h incubation, 20 μ l MTT (5 mg/ml) was added to each well and incubated at 37°C for 4 h. Subsequently, the culture medium was removed, and 150 μ l DMSO was added to each well. After shaking for 10 min, the 96-well plate was read on an enzyme-labeled device at 490 nm to calculate the cell viability rate.

Statistical analysis

All results are shown as the mean \pm standard deviation. GraphPad Prism 5 software was used to conduct statistical analyses. Student's t test or one-way analysis of variance was used to determine significant difference. $P < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) were statistically significant. All experiments were repeated independently at least three times.

Results

DPEP1 is upregulated in COAD, and correlates with poor DSS and PFI in COAD patients

To explore the possible roles of DPEP1 in tumors, we first analyzed its expression in TCGA and GTEx pancancer databases. As shown in Figure 1A, DPEP1 expression was higher in BRCA, CHOL, COAD, DLBC, ESCA, GBM, HNSC, LAML, LGG, LIHC, LUAD, READ, STAD and THYM, than in normal tissues. We also found that DPEP1 mRNA expression was significantly higher in colon cancer tissues than in adjacent normal colon tissues (Figure 1B-1C). To further evaluate the expression of DPEP1 protein in COAD, immunohistochemistry (IHC) images were downloaded from The Human Protein Atlas database to analyze the DPEP1 protein staining, intensity, quantity and location. We found that the DPEP1 protein in colon cancer tissues was higher than that in normal colon tissues (Figure 1D-1E). Survival analysis indicated that the high expression level of DPEP1 negative correlated with DSS and PFI, but was not significantly correlated with the overall survival (Figure 1F-1H).

Next, survival analysis for DPEP1 across the cancers was performed with the best cutoff value for DPEP1 mRNA expression. The high expression of DPEP1 in ACC, BLCA, LGG, SARC, STAD, and UCS was associated with an unfavorable overall survival, but HNSC, KIRC, KIRP, SKCM, and THYM patients with higher expression of DPEP1 had better overall survivals (Supplementary Figure 1).

Correlation and enrichment analysis

To further explore the functions and pathways associated with DPEP1, we performed a correlation analysis between DPEP1 and all other mRNAs in colon cancer using TCGA data (Supplementary Table 2). The 200 most positively associated genes with DPEP1 were selected for enrichment analysis, and the 50 most common genes are shown in a heat map (Figure 2). GO enrichment analysis showed that DPEP1 was mainly associated with regulating the Wnt signaling pathway, somite development, vacuolar membrane, apical plasma membrane, phospholipid binding, and modified amino acid binding (Supplementary Figure 2A). KEGG signaling pathway analysis showed that the Wnt signaling pathway, Hippo signaling pathway, and peroxisome were mainly enriched (Supplementary Figure 2B).

Differential expression analysis and Venn analysis

The TCGA-COAD dataset and GSE74602 dataset were subjected to differential expression analysis with an adjusted p value < 0.05 and $|\log_2 \text{fold change (FC)}| > 1.5$, respectively (Figure 3A-3B, Supplementary Table 3-4). The top 100 genes that were most positively associated with DPEP1 obtained from correlation analysis, upregulated genes in TCGA-COAD and upregulated genes in GSE74602 were selected for Venn analysis. Subsequently, ASCL2, RNF43, LY6G6D, and AXIN2 were obtained (Figure 3C, Supplementary Table 5). Among them, ASCL2 was the most upregulated gene in colon cancer. Furthermore, the GEPIA database was used to further verify the correlation between DPEP1 and ASCL2. The results showed that DPEP1 and ASCL2 had a strong positive correlation (Figure 3D).

Correlation between immune cell infiltration and the expression of DPEP1 or ASCL2 in COAD

Immune cell infiltration plays an important role in patients treated with colon cancer. Recently, Xueting Ren found that a gene signature containing DPEP1 affected the immune infiltration in KIRC[16]. We found that DPEP1 and ASCL2 had a strong positive correlation. Thus, the TIMER and GSCA databases were used to explore whether the expression of DPEP1 and ASCL2 was correlated with immune infiltration in colon cancer. The results showed that DPEP1 expression was negatively correlated with the levels of B cells, CD8+ T cells, Th17 cells, and Th2 cells (Figure 4A, Table 1). The expression of ASCL2 was negatively correlated with the infiltrating levels of B cells, CD8+ T cells, macrophages, Th17 cells and Th2 cells (Figure 4B, Table 1). Furthermore, PD1 and PD-L1 are important immune checkpoints that are related with tumor immune escape. The correlation between ASCL2 or DPEP1 and PD1/PD-L1 was assessed. Two different databases analyses, TIMER and GEPIA databases, have similar results that there was significant negative correlation of ASCL2, or DPEP1 with PD1, or PD-L1 (Figure 4C-4F). Taken together, these results show that DPEP1 and ASCL2 may play a role in immune infiltration of the colon cancer microenvironment.

Table 1
Correlation analysis between ASCL2 or DPEP1 and immune cells in COAD determined by the GSCA database.

Immune cell	ASCL2			DPEP1		
	R value	p value	FDR	R value	p value	FDR
B cell	-0.14	9.02E-03	1.87E-02	-0.20	1.81E-04	5.80E-04
CD8 ⁺ T cell	-0.27	4.30E-07	4.78E-06	-0.30	2.48E-08	3.78E-07
CD4 ⁺ T cell	-0.19	6.62E-04	1.37E-03	-0.21	1.59E-04	3.62E-04
Macrophage	-0.20	1.84E-04	7.41E-04	-0.11	4.99E-02	9.68E-02
Neutrophil	0.30	1.96E-08	1.24E-07	0.23	3.67E-05	1.38E-04
Dendritic cell	-0.10	6.91E-02	1.36E-01	-0.01	8.62E-01	9.10E-01
Th1	-0.07	2.00E-01	3.29E-01	-0.11	5.18E-02	1.18E-01
Th17	-0.25	2.92E-06	2.26E-05	-0.18	8.86E-04	3.11E-03
Th2	-0.39	1.11E-13	8.64E-13	-0.41	8.00E-15	7.24E-14

The stability of the ASCL2 protein was maintained by DPEP1 in colon cells.

Bioinformatics analysis showed a significant positive correlation between ASCL2 and DPEP1. Thus, immunohistochemistry and Western blotting were conducted to validate DPEP1 and ASCL2 protein levels in colon cancer and adjacent tissues. The results suggested that compared to normal colon tissue, both of the DPEP1 and ASCL2 protein levels were upregulated in colon cancer (Figure 5A-5C). In addition, ASCL2 was upregulated when DPEP1 was overexpressed in HCT116 cells but downregulated when DPEP1 was knocked-down in SW620 cells (Figure 5D-5E). Moreover, Co-immunoprecipitation experiments proved that DPEP1 and ASCL2 proteins can bind to each other in HCT116 and SW620 cells (Figure 5F-5G). To study how DPEP1 upregulates ASCL2, HCT116 and SW620 cells were treated with CHX (a protein synthesis inhibitor) for different periods to arrest protein synthesis, and the degradation rate constant of ASCL2 protein was analyzed by Western blotting. Compared with the control groups, DPEP1 overexpression in HCT116 cells lengthened the half-life of the ASCL2 protein, whereas DPEP1 interference in SW620 cells accelerated ASCL2 degradation (Figure 5H-5I). MG132 (a proteasome inhibitor) was used to inhibit the ubiquitin-proteasomal degradation pathway in cells. HCT116 cells were treated with MG132 for 1, 2, 4, or 6 h. Subsequently, the ASCL2 protein was tested by Western blotting. The results showed that the increase in ASCL2 protein levels in HCT116 cells treated with MG132 was time-dependent, indicating that the degradation of the ASCL2 protein was regulated by the ubiquitin pathway (Figure 5J). Furthermore, to study the effect of DPEP1 expression on the ubiquitination of ASCL2 proteins, HCT116 cells were transiently transfected with pCMV or pCMV-DPEP1. Immunoprecipitation was used to pull-down ASCL2 proteins. Subsequently, the ubiquitination levels of ASCL2 proteins were tested by immunoblotting ubiquitin. The results indicated that DPEP1

overexpression in HCT116 cells increased ASCL2 protein levels by inhibiting ubiquitin pathway degradation (Figure 5K). In summary, DPEP1 expression increases the stability of ASCL2 proteins in colon cancer cells.

DPEP1 expression was upregulated by ASCL2 in colon cancer cells

To explore whether DPEP1 can be regulated by ASCL2, HCT116 and SW480 cells were transiently transfected with pcDNA3 or pcDNA3-ASCL2 plasmids, and SW620 cells were transiently transfected with siRNA (NC), siASCL2-1, or siASCL2-2. The results suggested that the mRNA and protein expression levels of DPEP1 were upregulated when ASCL2 was overexpressed in HCT116 and SW480 cells but downregulated when ASCL2 was knocked down in SW620 cells (Figure 6A-6D). Furthermore, the activities of the DPEP1 promoter were detected by dual luciferase reporter experiments. The results indicated that the activities of the DPEP1 promoter were upregulated when ASCL2 was overexpressed in HCT116 cells but downregulated when ASCL2 was knocked down in SW620 cells (Figure 6E-6F). In summary, ASCL2 can affect the activities of the DPEP1 promoter, which may cause an increase in the mRNA and protein expression levels of DPEP1.

DPEP1 increased colon cancer cells drug resistance in an ASCL2 dependent manner

Previous studies have proved that ASCL2 is a colon stem cell marker[17, 18]. Our studies found that ASCL2 and DPEP1 are positively correlated and can be combined with each other. Thus, in order to explore whether DPEP1 can affect the stemness of colon cancer cells, Western blot was used to test stem-related proteins, including ASCL2, CD133, CD44, LGR5, and NKD1. The results suggested that DPEP1 can increase the expression levels of ASCL2, CD133, CD44, LGR5, and NKD1(Figure 7A). Functionally, cell stemness can increase the resistance of anti-tumor drugs[19]. Thus, we performed chemotherapy drug resistance experiment. HCT116 cells were transiently transfected with pCMV, pCMV-DPEP1, and pCMV-DPEP1 + siRNA-ASCL2 (100 nM). The results showed that DPEP1 overexpression inhibited the death of HCT116 cells that were incubated with oxaliplatin or irinotecan, whereas knockdown of ASCL2 significantly reversed this phenomenon (Figure 7B-7E). These results indicated that DPEP1 increased the drug resistance of colon cancer cells in an ASCL2 dependent manner.

Discussion

Increasing evidence has suggested that DPEP1 is a differentially expressed gene in various tumors, but the biological function of DPEP1 in multiple tumors is still controversial. For example, DPEP1 was reduced in pancreatic ductal adenocarcinoma, inhibited the invasiveness of tumor cells, enhanced chemosensitivity and predicted a better clinical outcome[20]. Moreover, DPEP1 acts as a tumor suppressor gene in breast cancer[21]. In contrast, DPEP1 was highly expressed in leukemia cells,

hepatoblastoma cells and colon cancer cells and promoted tumor progression[6, 7, 10–12], suggesting that DPEP1 might act as an oncogene in these cancers. Our study found that DPEP1 mRNA and protein expression levels in colon cancer were higher than those in normal colon tissues, which was consistent with related studies[8, 11, 12]. Bioinformatics analysis found that DPEP1 expression was negatively correlated with DSS and PFI in colon cancer patients but had no statistical significance with OS. However, P A Eisenach et al. found that the high expression group of DPEP1 had worse OS. This may be related to the sample size and statistical methods.

Our correlation analysis, enrichment analysis, differential expression analysis and Venn analysis showed that DPEP1 and ASCL2 were significantly positively correlated and likely related to the Wnt signaling pathway and Hippo signaling pathway. Recent studies have shown that the Hippo signaling pathway regulates innate immunity in HIV-1 infection or hepatitis B Virus infection and correlates with immune infiltration in lung squamous cell carcinoma[22–24]. Furthermore, the Wnt signaling pathway was also associated with immune infiltration in glioblastomas or ovarian cancer[25, 26]. Therefore, we further investigated the correlation of DPEP1 or ASCL2 expression with immune cell infiltration in colon cancer. The TIMER and GSCA databases suggested that DPEP1 and ASCL2 were both negatively related to B cells, CD8+ T cells, Th17 cells and Th2 cells, suggesting a key role of DPEP1 and ASCL2 in regulating tumor immunology.

Next, we found a positive correlation between DPEP1 and ASCL2 expression in clinical colon cancer tissues and colon cancer cell lines. Furthermore, DPEP1 upregulated ASCL2 protein levels but not its mRNA levels in colon cancer cells and suppressed ASCL2 protein degradation in CHX (a protein synthesis inhibitor) chase experiments. These data indicate that DPEP1 can enhance ASCL2 expression at the posttranslational level. In turn, ASCL2, a transcription factor, upregulated the mRNA and protein levels of DPEP1. Finally, the MTT assays indicated that DPEP1 increased drug resistance in colon cancer cells in an ASCL2-dependent manner.

Conclusion

In summary, ASCL2 upregulated DPEP1 expression levels, and DPEP1 expression restrained ASCL2 protein degradation, which may explain why DPEP1 increased drug resistance in colon cancer cells in an ASCL2-dependent manner. Furthermore, DPEP1 and ASCL2 were both correlated with immune cell infiltration.

Abbreviations

ACC
adrenocortical carcinoma
BLCA
bladder urothelial carcinoma
BRCA breast

invasive carcinoma
CESC
cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL
cholangiocarcinoma
COAD
colon adenocarcinoma
DLBC
lymphoid neoplasm diffuse large B-cell lymphoma
ESCA
esophageal carcinoma
GBM
glioblastoma multiforme
HNSC
head and neck squamous cell carcinoma
KICH
kidney chromophobe
KIRC
kidney renal clear cell carcinoma
KIRP
kidney renal papillary cell carcinoma
LAML
acute myeloid leukemia
LGG
brain lower grade glioma
LIHC
liver hepatocellular carcinoma
LUAD
lung adenocarcinoma
LUSC
lung squamous cell carcinoma
OV
ovarian serous cystadenocarcinoma
PAAD
pancreatic adenocarcinoma
PRAD
prostate adenocarcinoma
READ
rectum adenocarcinoma
SARC

sarcoma
SCKM
skin cutaneous melanoma
STAD
stomach adenocarcinoma
TGCT
testicular germ cell tumor
THCA
thyroid carcinoma
THYM
thymoma
UCEC
uterine corpus endometrial carcinoma
UCS
uterine carcinosarcoma
TCGA
The Cancer Genome Atlas
KEGG
Kyoto Encyclopedia of Genes and Genomes
GO
Gene ontology

Declarations

Acknowledgements

The authors gratefully acknowledge the multiple databases, which made the data available.

Authors' contributions

All authors participated in the conception and design of the study; Protocol/project development—JJ and QL; Data collection or management —YS, QZ, CY, YW, WL; Data analysis—CZ, JL, YD, GQ; Manuscript writing/editing—CZ; All authors read and approved the paper.

FUNDING

The work was supported by National Natural Science Foundation of China, [grant number 81872275]; by Scientific Research Project of Jiangsu Commission of Health [M2020002]; by the funds of Changzhou Sci & Tech Program, [grant number CJ20200004]; by Wujin Sci & Tech Program [WS201924, WS201922]; by Open Project of Jiangsu Provincial Key Laboratory of Tumor Biotherapy [XZSYSKF2020005].

Availability of data and materials

The GDC TCGA Colon Cancer (COAD) dataset (<https://xenabrowser.net/>) and The Gene Expression Omnibus (GEO) dataset (<https://www.ncbi.nlm.nih.gov/geo/>) are available by contacting the author.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Wujin Hospital affiliated with Jiangsu University. Informed consents were obtained from the patients before the study.

Consent for publication

Not applicable.

Competing interests

Authors of this article declare that they have no conflict of interest.

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Figures

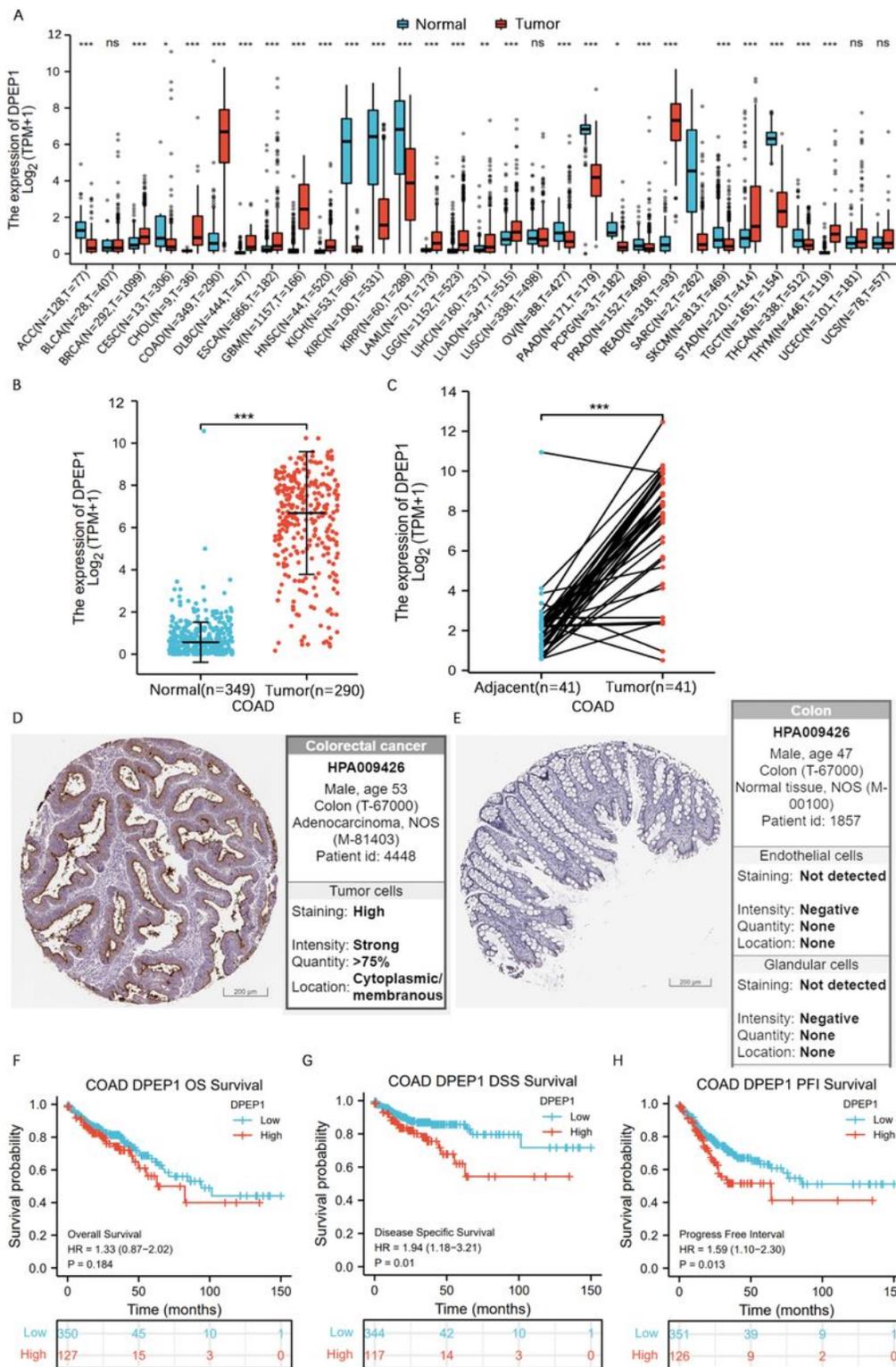


Figure 1

DPEP1 is upregulated in COAD and high DPEP1 expression correlates with poor DSS and PFI in COAD patients. (A) DPEP1 expression in tumor and normal tissues in TCGA and GTex pancancer data. (B) DPEP1 expression in normal and tumor tissues in COAD from TCGA and GTex. (C) Expression of DPEP1 in paired normal and tumor tissues in the COAD from TCGA. (D) Immunohistochemical image showing DPEP1 expression in the tissues of colon adenocarcinoma from the Human Protein Atlas. (E)

Immunohistochemistry image showing DPEP1 expression in the normal colon tissues from the Human Protein Atlas. (F) The correlation between DPEP1 expression and overall survival in COAD was analyzed using the TCGA database with the best cutoff value for DPEP1 mRNA expression. (G) The correlation between DPEP1 expression and disease-specific survival in COAD was analyzed using TCGA database with the best cutoff value of DPEP1 mRNA expression. (H) The correlation between DPEP1 expression and the progression-free interval in COAD was analyzed using the TCGA database with the best cutoff value of DPEP1 mRNA expression. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns refers to no statistical significance. Data were shown as the mean \pm SD.

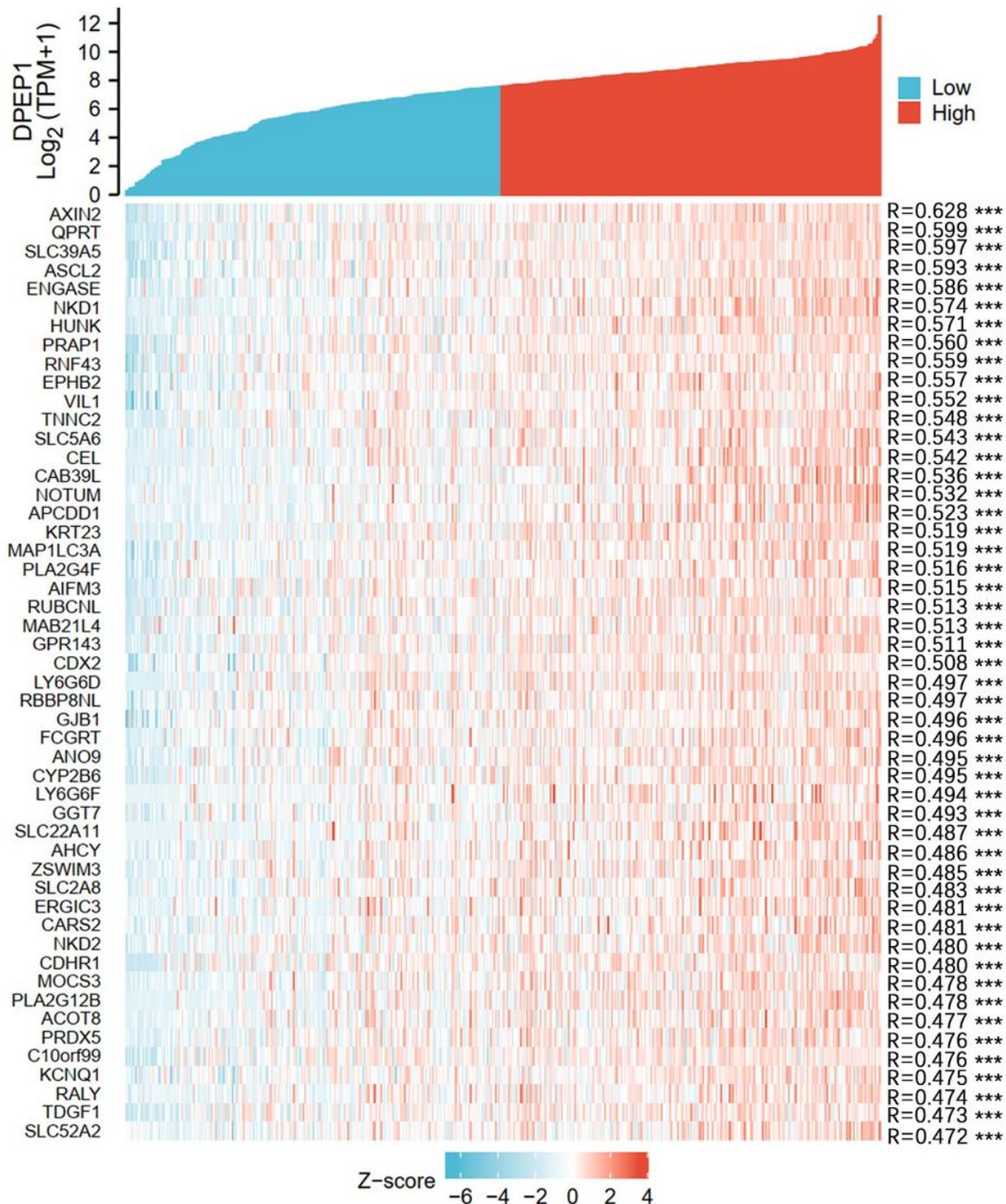


Figure 2

The correlation analysis of DPEP1. The top 50 genes most positively associated with DPEP1 are shown in a heatmap. Data were normalized using the Z-score standardization method. *** $p < 0.001$.

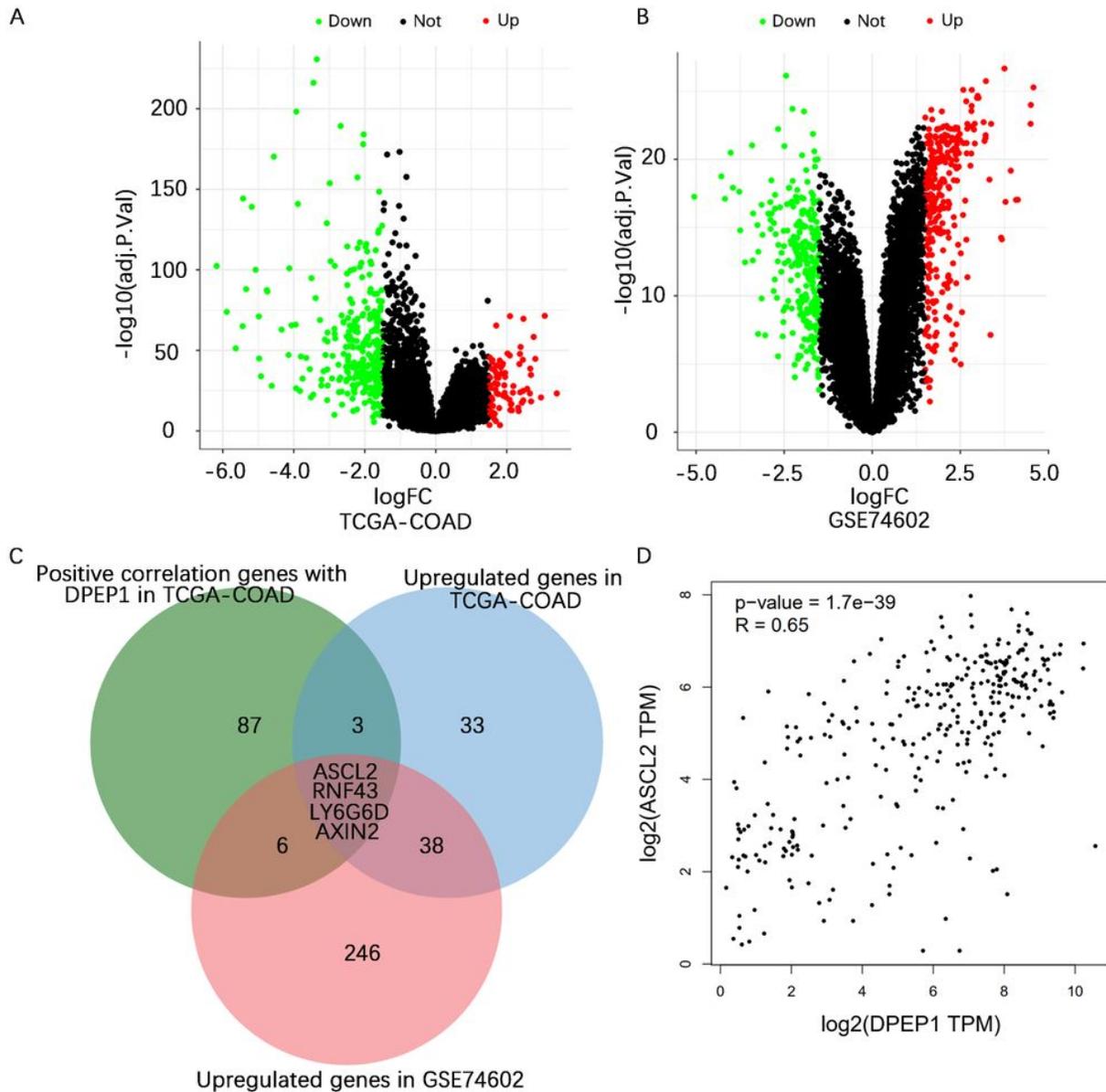


Figure 3

Differential expression analysis and Venn analysis. (A) Volcano plots of the distribution of DEGs in TCGA-COAD. (B) Volcano plots of the distribution of DEGs in GSE74602. (C) Venn analysis of the upregulated genes in TCGA-COAD, the upregulated genes in GSE74602 and the top 100 most positively correlated genes with DPEP1 in TCGA-COAD. (D) Correlation analysis between DPEP1 expression and ASCL2 expression based on the GEPIA online site using TCGA-COAD tumor and normal datasets. DEG, differentially expressed genes; GEPIA, gene expression profiling interactive analysis.

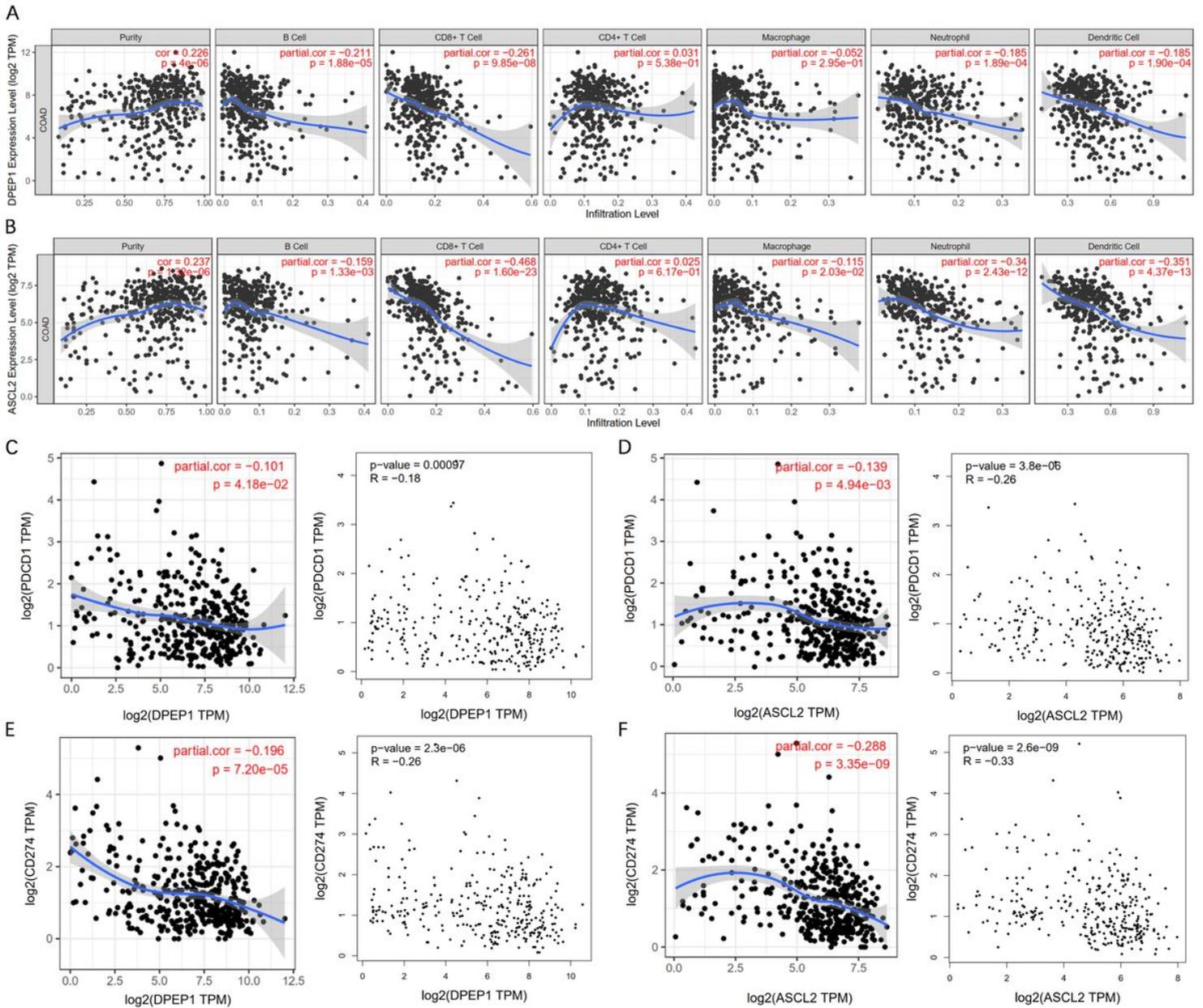


Figure 4

Correlation of DPEP1 or ASCL2 expression with immune cell infiltration in COAD (colon adenocarcinoma). (A) The expression of DPEP1 is significantly positively related to tumor purity and has significant negative correlations with the infiltrating levels of B cells, CD8+ T cells, neutrophils, and dendritic cells in COAD. (B) ASCL2 expression is significantly positively related to tumor purity and has significant negative correlations with infiltrating levels of B cells, CD8+ T cells, macrophages, neutrophils, and dendritic cells in COAD.

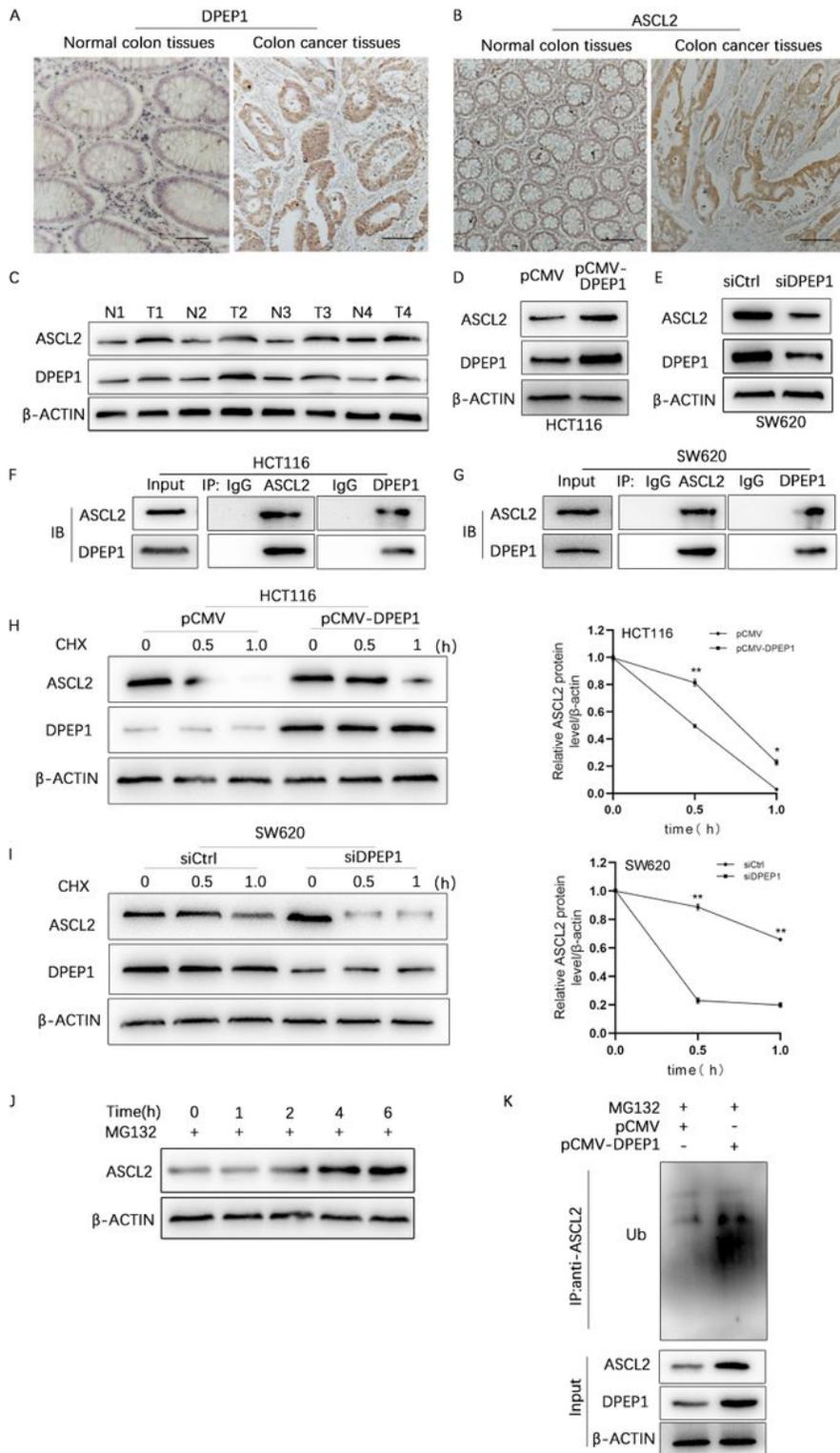


Figure 5

ASCL2 protein stability was maintained by DPEP1 in colon cells. (A-B) Immunohistochemical analysis of DPEP1 and ASCL2 in the colon cancer and adjacent tissues. (C) Western blot analysis of DPEP1 and ASCL2 in colon cancer tissues and adjacent tissues. (D-E) Western blot analysis of DPEP1 and ASCL2 in HCT116 cells and SW620 cells. (F-G) Co-immunoprecipitation analysis of DPEP1 and ASCL2 in HCT116 cells and SW620 cells. (H) The level of ASCL2 proteins in HCT116 cells and HCT116-DPEP1 cells treated

with CHX (15 mg/ml) for 0, 0.5 and 1.0 h was tested by Western blot. The ASCL2 intensity normalized to β -actin was plotted. (I) The level of ASCL2 proteins in SW620 cells transiently transfected with negative control (NC) siRNA or DPEP1 siRNA (100 nM) treated with CHX (15 mg/ml) for 0, 0.5 and 1.0 h was tested by Western blot. The ASCL2 intensity normalized to β -actin was plotted. (J) ASCL2 protein levels in HCT116 cells treated with DMSO or MG132 (10 mM) for 1, 2, 4 or 6 h, were measured by Western blot. (K) Degradation of ASCL2 proteins in HCT116 cells transiently transfected with pCMV (1.0 mg) or pCMV-DPEP1 (1.0 mg) was measured by Western blot.

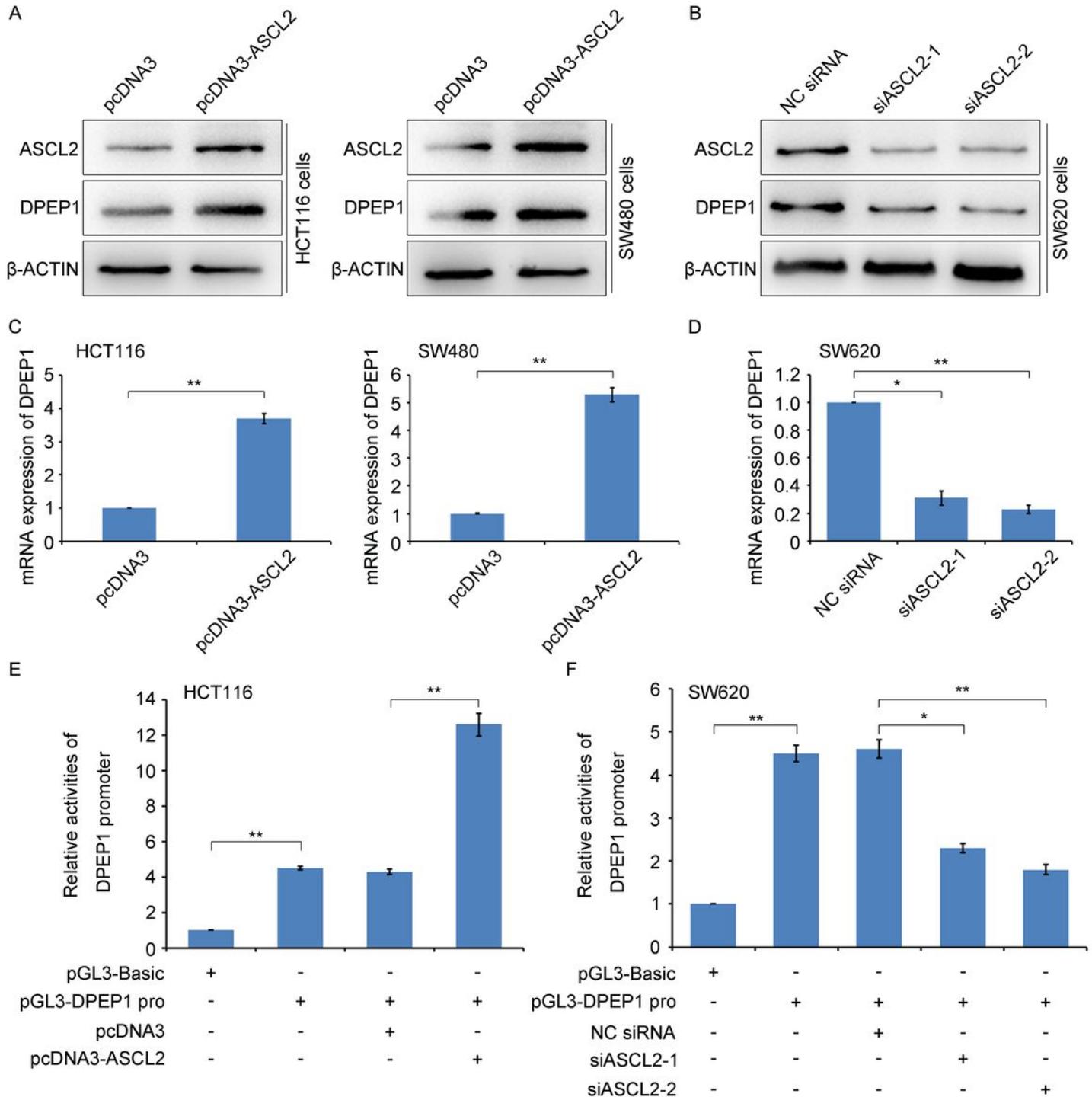


Figure 6

The expression level of DPEP1 was upregulated by ASCL2 in colon cancer cells. (A) Western blotting was used to detect the protein levels of DPEP1 and ASCL2 in HCT116 and SW480 cells transiently transfected with pcDNA3 or pcDNA3-ASCL2 plasmids, respectively. (B) Western blotting was used to detect the protein levels of DPEP1 and ASCL2 in SW620 cells that were transiently transfected with siRNA (NC), siASCL2-1, or siASCL2-2. (C) The mRNA expression level of DPEP1 was tested by qRT-PCR in HCT116 and SW480 cells that were transiently transfected with pcDNA3 or pcDNA3-ASCL2 plasmids. (D) The mRNA expression level of DPEP1 was tested by qRT-PCR in SW620 cells transiently transfected with siRNA (NC), siASCL2-1, or siASCL2-2. (E) Relative activities of the DPEP1 promoter were detected by dual luciferase reporter experiments in HCT116 cells that were transiently transfected with pcDNA3 or pcDNA3-ASCL2 plasmids. (F) Relative activities of the DPEP1 promoter were detected by dual luciferase reporter experiments in SW620 cells that were transiently transfected with siRNA(NC), siASCL2-1, or siASCL2-2.

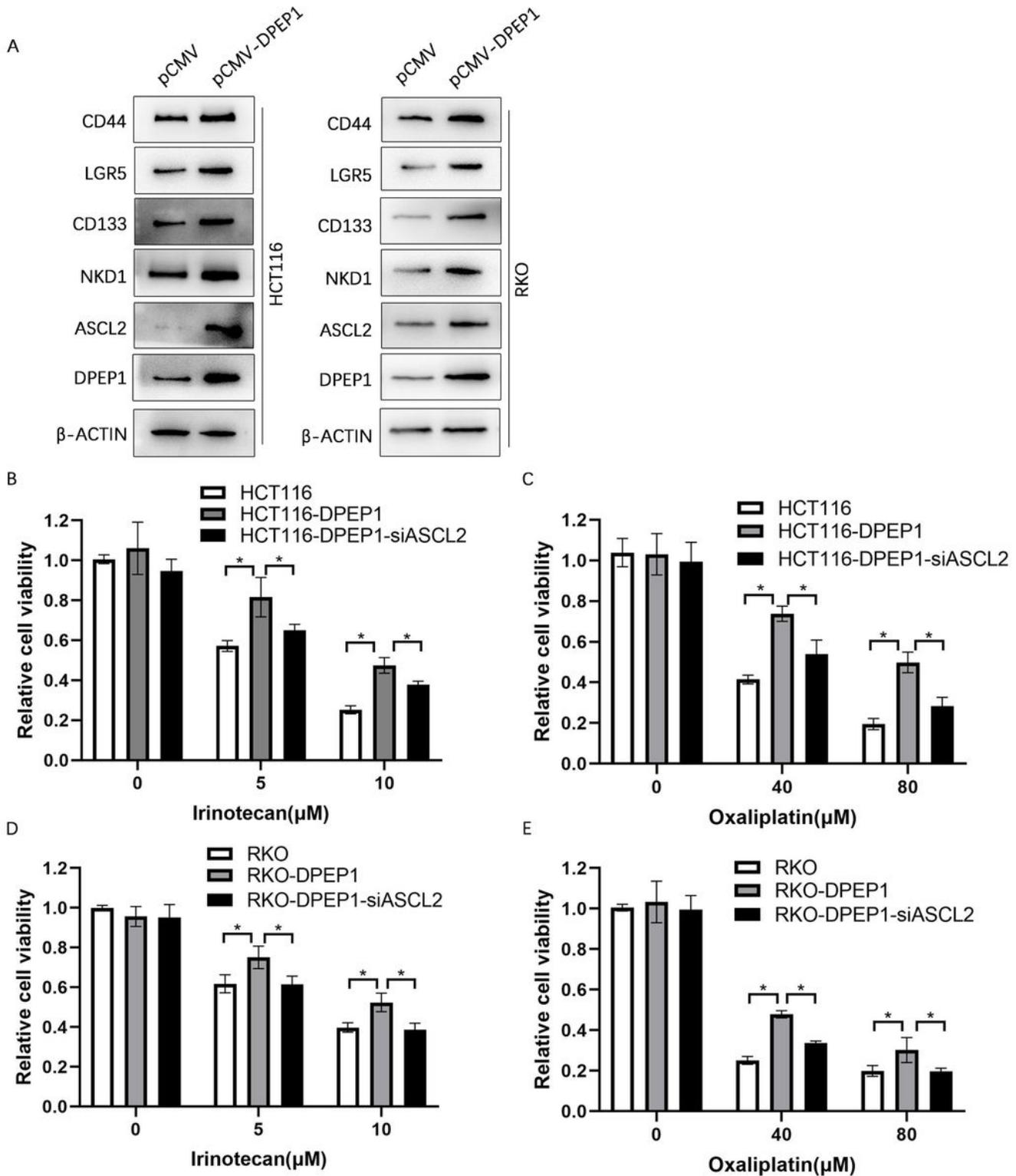


Figure 7

DPEP1 improves chemoresistance. (A) Western blot analysis of DPEP1 and cell stem related proteins (ASCL2, CD44, CD133, LGR5, and NKD1) in HCT116 and RKO cells. (B-C) pCMV, pCMV-DPEP1 and pCMV-DPEP1+siASCL2 were transiently transfected into HCT116 cells and then each group of cells was treated with irinotecan or oxaliplatin for 48 h. (D-E) pCMV, pCMV-DPEP1 and pCMV-DPEP1+siASCL2 were

transiently transfected into RKO cells and then each group of cells was treated with irinotecan or oxaliplatin for 48 h. The cell viability was determined using MTT.

Supplementary Files

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