S100A4 promotes colorectal carcinoma growth by enhancing aerobic glycolysis

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Abstract

Background Glucose metabolism transformation plays critical role in cancer cell malignancies maintenance. Aberrant cancer cell metabolism is considered to be the hallmark of cancer. S100A4 has been identified as an oncogene in a variety of cancers. However, its role in the cancer cell glucose reprogramming has been seldom reported. The aim of this study was to examine the role of S100A4 in aerobic glycolysis in colorectal cancer (CRC).

Methods We investigated S100A4 expression in 224 cases of primary CRC and matched normal colonic tissue specimens, and explored the underlying mechanisms of altered S100A4 expression as well as the impact of this altered expression on CRC growth and glycolysis using in vitro and animal models of CRC.

Results S100A4 was more highly expressed in CRC tissues than in the adjacent normal tissues (59.4% vs 17.4%, P <0.05). Higher S100A4 expression was associated with advanced node stage (P =0.018) and larger tumor size (P =0.035). A Cox proportional hazards model suggested that S100A4 expression was an independent prognostic factor for both OS (HR: 3.967, 95%CI: 1.919-8.200, P <0.001) and DFS (HR: 4.350, 95%CI: 2.264-8.358, P <0.001) in CRC after surgery. Experimentally, silencing S100A4 expression significantly decreased the growth and glycolysis rate of CRC both in vitro and in vivo . Mechanically, S100A4 could affect the hypoxia-inducible factor (HIF)-1α activity as demonstrated by the HIF-1α response element−luciferase activity in CRC cells.

Conclusions These results disclose a novel role for S100A4 in reprogramming the metabolic process in CRC by affecting the HIF-1α activity and provide potential prognostic predictors for CRC.

Background

With more than one million newly diagnosed cases and half a million deaths every year worldwide, colorectal cancer (CRC) is a major public health threaten in both developed and developing countries[1]. Despite advances in surgical intervention as well as chemotherapy, the CRC outcome remains poor due to high metastatic potential. TNM staging is one of the main predictors for prognosis of CRC, but even patients with the same disease stage have quite different prognosis. Hence, additional biomarkers for predicting disease are urgently needed to define which can be potentially used for diagnosis and as therapeutic targets in CRC.

Aerobic glycolysis, also known as Warburg effect, is characterized as the continuous conversion of glucose to lactate even in the presence of oxygen and is a distinctive hallmark of solid tumors, including colorectal cancer [2, 3]. Because it is closely related with increased progression and metastasis abilities of cancer, aerobic glycolysis is one of the basic characteristics of invasive tumors [4, 5]. Recently, studies have revealed that genetic or epigenetic changes in oncogenes or tumor suppressors lead to activation of glycolysis, as most glycolytic enzymes are regulated by oncogenes or tumor suppressors such as hypoxia inducible factor (HIF)[6], Myc[7], FOXC1[8], FOXM1[9], Gas1[5], et al.

S100A4 belongs to the S100 family of proteins and has been reported to be involved in diverse biological processes, including migration, invasion, metastasis and prognosis of various tumors [10, 11]. Studies have revealed that pathophysiological progression is related with S100A4 expression in lung cancer [12], CRC [13], breast cancer[14], prostate cancer[15], and melanoma[16], which is closely related to tumor morbidity and metastasis. Of note, recent evidence indicates that S100A4 alters metabolism and promotes invasion of lung cancer cells by up-regulating mitochondrial complex I protein NDUFS2 [17]. However, the underline mechanism of S100A4 in CRC is not fully investigated.

Here, we aimed to explore the role of S100A4 in mediating glucose reprogram in CRC. Interestingly, we found that augmented expression of S100A4 was frequently detected in CRC and was significantly associated with a poor outcome. Furthermore, we found that S100A4 facilitated aerobic glycolysis in CRC cancer cells via enhanced hypoxia-inducible factor-1α activity.

Materials And Methods

Patient samples

Primary CRC and adjacent non-tumor colonic tissue specimens were collected immediately after surgical removal and stored at -80˚C. An additional 224 paraffin-embedded CRC tissues and adjacent non-tumor tissues were obtained from our institutes between 2010 and 2012, and were randomly selected for immunohistochemistry (IHC) study. The pathological diagnoses were confirmed by at least two pathologists and restaged according to the 8th American Joint Committee on Cancer (AJCC) guidelines. The study protocol and consent form were approved by the Institutional Ethics Committee of the Taizhou Municipal Hospital, Medical College of Taizhou University. All patients were informed of the aims of the study and provided written informed consent.

IHC analysis

IHC study was used to explore S100A4 expression in patients as previously described [8]. Primary anti-S100A4 antibody (ab124805, 1:1000; Abcam) and goat anti-rabbit Envision System Plus-HRP (Dako Cytomation) were used for the study. Phosphate-buffered saline was used as a negative control. Data were assessed by two independent pathologists. A semiquantitative scoring system [18] was used to evaluate both staining intensity and the percentage of stained cells.

RNA isolation and qPCR assay

Total RNA was extracted from CRC and adjacent non-tumor tissues using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. First strand cDNA was then synthesized using the PrimeScript™ RT Master Mix (Perfect Real Time) kit (RR036A, Takara). The qPCR assay was conducted in a 10 µl reaction mixture according to the SYBRGreen Master Mix (Roche, Mannheim, Germany) and analyzed in a 96-well plate using the Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as previous described[8, 19]. All primers were

listed in Table 1.β-actin was used as an internal control. Relative values of transcripts were calculated using the formula: $2^{-\Delta\Delta Ct}$. Experiments were performed in triplicate.

Table 1

Association between S100A4 expression and clinicpathological factors in colorectal cancers (n = 224)

Variable	n	S100A4 Expression		χ^2 Value	P value
		Low	High		
Gender				0.027	0.870
Male	129	52(58.2)	76(57.1)		
Female	95	38(41.8)	57(42.9)		
Age				0.002	0.965
≦ 60	135	55(60.4)	80(60.2)		
> 60	89	36(39.6)	53(39.8)		
Tumor location				0.853	0.356
Colon	100	44(48.4)	56(42.1)		
Rectum	124	47(51.6)	77(57.9)		
Grade				1.252	0.535
Well	63	23(25.2)	40(30.1)		
Moderate	113	50(54.9)	63(47.4)		
Poor	48	18(19.8)	30(22.6)		
T category				7.160	0.067
T1	6	5(5.5)	1(0.8)		
T2	35	18(19.8)	17(12.8)		
T3	93	35(38.5)	58(43.6)		
T4	90	33(36.3)	57(42.9)		
N stage				8.001	0.018
N0	106	53(58.2)	53(39.8)		
N1	71	25(27.5)	46(34.6)		
N2	47	13(14.3)	34(25.6)		
Tumorsize(cm)				4.444	0.035

[a] Fisher's exact test

Variable	n	S100A4 Expression		χ² Value	P value
≤ 5	160	72(79.1)	88(66.2)		
> 5	64	19(20.9)	45(33.8)		
Lymphovascular invasion				12.955	< 0.001
Negative	178	83(91.2)	95(71.4)		
Positive	46	8(8.8)	38(28.6)		
Perineural invasion				2.798	0.094
Negative	194	83(91.2)	111(83.5)		
Positive	30	8(8.8)	22(16.5)		
CEA				3.647	0.056
Normal	146	66(72.5)	80(60.2)		
High	78	25(27.5)	53(39.8)		
a Fisher's exact test					

Western blotting

Standard Western blotting was carried out as previous described [8, 20]. Primary antibodies anti-S100A4 (ab124805, 1:1000; Abcam), or anti-β-actin (ab133626, 1:5000; Abcam) and secondary antibodies (anti-rabbit IgG; Cell Signaling Technology, Danvers, MA, USA) were used. Equal-amount protein sample loading was monitored using an anti-β-actin antibody.

Glucose uptake and lactate production assays

The glucose assay kit (BioVision, Milpitas, CA, USA) was used to detect relative glucose uptake and lactate production among different S100A4 expression groups in CRC cells. All reactions were performed in triplicate as described previously [8].

Cell proliferation and clonogenic assay

Colon cancer cell proliferation was determined using CCK-8 assay according to the manufacturer's instructions. To determine cells' clonogenic ability, they were transplanted into a six-well dish at a density of 200 cells per well and cultured for 10−14 days and form colonies. The cells were then stained with 0.1% crystal violet after being fixed with methanol. All of the visible colonies were counted manually.

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)

Cellular mitochondrial function and glycolytic capacity were measured using the Seahorse Bioscience XF96 Extracellular Flux Analyzer as previous described [20]. Briefly, cells were plated in XF96 Cell Culture Microplates (Seahorse Bioscience) at an initial cellular density of 4×10^4 cells per well overnight. Seahorse buffer consists of DMEM medium, phenol red, 25 mM glucose, 2 mM sodium pyruvate, and 2 mM glutamine. For ECAR measurement, 10 mM glucose, 1 µM oligomycin, and 100 mM 2-deoxy-glucose (2-DG) were automatically added to measure ECAR value. After monitoring baseline respiration, 1 µM oligomycin, 1 µM FCCP, and 1 µM rotenone were automatically injected into XF96 Cell Culture Microplates to measure the OCR. The ECAR and OCR values were calculated after normalization of cell number. All experiments were performed in triplicate.

Luciferase assay

Cells were incubated at 37 °C overnight in 96-well plates. Each group was co-transfected with HRE promoter reporters, shS100A4 or pcDNA3.1-S100A4 plasmid. Forty-eight hours later, the cells were analyzed using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) as manufacturer's description.

Xenografted nude mice model and PET/CT study

All procedures involving mice were conducted in accordance with Taizhou Municipal Hospital, Medical College of Taizhou University Animal Care guidelines. Five 4–8 weeks old male Balb/C athymic nude mouse were prepared for the study. RKO-shS100A4 or RKO-scramble cells were injected subcutaneously into the right/left forelimbs of nude mice. After 4 weeks, all the injected mice were euthanatized. Tumor xenografts were harvested and weighted. Tumor volume (TV) was calculated weekly for 4 weeks according to the formula: TV (mm^3) = length × width^2 × 0.5[5].

For PET/CT study, the mice were starved for 8 h, then given 6 µCi 18F-FDG per gram body weight and undertook PET/CT scan 1 h later.

Statistical analysis

The χ2 test or Fisher's exact test was used to analyze the correlation between S100A4 expression and clinicopathological characteristics. The survival rates were calculated by the Kaplan–Meier method and the differences between the survival curves were determined by the log-rank test. The significant variables in the univariate analyses were incorporated into the multivariate analysis. The HR with 95% confidence interval (CI) was measured to estimate the hazard risk of individual factors.

Data from in vitro experiments are presented as the mean ± SD. The significance of the in vitro and in vivo data was tested using the Student t-test (two-tailed) or one-way ANOVA. A two-sided P-value of < 0.05 was considered to indicate statistical significance. Analyses were performed using the SPSS statistical software program version 17.0 (SPSS Inc., Chicago, IL).

Results

S100A4 expression patterns in CRC

To explore the expression pattern of S100A4 in CRC, we first used IHC to determine S100A4 protein expression in 224 paraffin embedded CRC tissues and paired adjacent non-tumor tissues. There were 132 male and 92 female cases with age ranging from 27 to 85 years (median 58). For CRC staging, we used the 8th American Joint Committee on Cancer (AJCC) stages as our criteria, there were 21 tumors at AJCC stage I, 83 at stage II, and 120 at stage III. Representative images of S100A4 IHC staining was shown in Fig. 1A. As expected, high expression of S100A4 was observed in 133 (59.4%) cases of CRC tissues, which was markedly higher than that in adjacent non-tumor tissues (17.4%) ($P<0.001$; Fig.1B). We then investigated S100A4 transcriptional expressions in 15 paired liver metastases, primary colon cancer, and adjacent normal colon tissues by RT-PCR analysis. S100A4 mRNA levels was found to be significantly different among the three groups. The most significant difference was between adjacent normal controls and liver metastases ($P< 0.001$; Fig.1C). The result indicated that S100A4 may promote the progression and metastasis of the CRC.

Clinical significance of S100A4 in CRC

As demonstrated in Table 1, the expression levels of S100A4 were closely associated with the lymph node involvement ($P=0.018$), lymphovascular invasion ($P=0.035$), and tumor size ($P<0.001$).

After a median follow-up period of 58 months (range 2-89 months), 74 out of 224 (33.0%) patients relapsed and 58 out of 224 (25.9%) died from the disease. The incidence of recurrences and deaths was significantly higher in patients with S100A4 overexpression group than those of low expression (both $P<0.001$). Kaplan-Meier survival analysis and log-rank test indicated that the expression of S100A4 was significantly correlated with overall survival OS ($\chi2=22.220$, $P<0.004$) and DFS ($\chi2=30.331$, $P<0.001$) of patients with CRC (Fig. 2A, 2B). More importantly, S100A4 was confirmed to be an independent predictor of both OS (HR: 3.967, 95%CI⊠1.919-8.200⊠$P<0.001$) and DFS (HR: 4.350, 95%CI⊠2.264-8.358⊠$P<0.001$) in multivariate regression analysis (Table 2).

S100A4 mediates cell growth and proliferation in CRC

Table 2 Univariate and multivariate analysis of survival in CRC

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
...vival				
Gender	1.011(0.601-1.700)	0.968		
Age	1.196(0.711-2.011)	0.500		
...umor location	0.777(0.465-1.301)	0.337		
Grade	1.104(0.760-1.603)	0.605		
T category	1.819(1.243-2.663)	0.002	1.327(0.897-1.963)	0.157
N stage	2.231(1.615-3.083)	<0.001	1.866(1.297-2.686)	0.001
Tumor size	2.312(1.373-3.893)	0.002	1.833(1.073-3.131)	0.027
...cular invasion	2.642(1.545-4.518)	<0.001	1.237(0.689-2.219)	0.476
...eural invasion	1.437(0.727-2.840)	0.297		
CEA	1.002(1.000-1.004)	0.040	1.009(0.582-1.751)	0.974
S100A4	4.710(2.312-9.596)	<0.001	3.967(1.919-8.200)	<0.001
...e Survival				
Gender	1.068(0.676-1.690)	0.777		
Age	1.072(0.674-1.705)	0.770		
...umor location	0.8649(0.548-1.365)	0.532		
Grade	1.168(0.840-1.624)	0.356		
T category	1.626(1.179-2.241)	0.003	1.227(0.881-1.710)	0.225
N stage	2.107(1.588-2.797)	<0.001	1.788(1.294-2.470)	<0.001
Tumor size	2.150(1.353-3.417)	0.001	1.857(1.150-2.997)	0.011
...cular invasion	2.609(1.617-4.212)	<0.001	1.295(0.774-2.165)	0.325
...eural invasion	1.826(1.036-3.220)	0.037	1.252(0.690-2.271)	0.460
CEA	1.430(0.901-2.270)	0.129		
S100A4	5.045(2.657-9.580)	<0.001	4.350(2.264-8.358)	<0.001

Abbreviation: CI, confidence interval; HR, hazard ratio

To assess the biological role of S100A4 in CRC, we constructed stable S100A4 knockdown cells in RKO and HCT116 cell lines by lentiviral transfection. Knockdown efficiency were determined by RT-PCR and western blotting (Figs.3A and 3B). The CCK-8 and colony forming assays were used to investigate whether S100A4 expression affected colon cancer cell proliferation *in vitro*. As shown in Fig.3C-D, there was a significantly decreased in the proliferation activity(Fig.3C) and colony forming ability(Figs.3D and 3E) of colon cancer cells after silencing S100A4 expression when compared with the control groups(*P*<0.05).

To further validate the results from the *in vivo* study, RKO-shS100A4 cells and RKO-scramble cells were subcutaneous injected into the bilateral subtemporal subcutaneous area of nude mice, respectively. After 4 weeks, the mice were sacrificed, the size and weight of the xenograft tumors were measured. The results demonstrated that the volume and weight of subcutaneous tumor derived from RKO-shS100A4 cells was significantly lower than that of its control group (*P*<0.05; Fig. 3F-3H).

S100A4 promotes aerobic glycolysis in colon cancer

It is well known that the growth of tumor requires glucose metabolism transformation to guarantee constant energy supply. Cancer cells undergo a process of energy metabolism remodeling from oxidative phosphorylation to less efficient glycolytic pathways in response to regional hypoxic stress. Under such stress, tumor cells rely mainly on glycolysis to maintain its malignant characteristics [2, 3]. To further understand the effect of S100A4 on glycolysis in colon cancer cells, we calculated changes in glucose utilization, lactate concentrations and ATP production of the stably transfected cells. As anticipated, silencing S100A4 expression significantly decreased the glucose utilization, lactate concentrations and ATP production in RKO and HCT116 cells (Fig. 4A-4C). Furthermore, we used Seahorse XF Extracellular Flux Analyzers to examine the impact of S100A4 on glycolysis, as reflected by extracellular acidification rate or ECAR. ECAR is an indicator of acidification of the medium surrounding cancer cells that caused by lactic acid, which is a product of aerobic glycolysis. The results demonstrated that the ECAR decreased significantly after silencing S100A4, reflecting the positive role of S100A4 in extracellular acidification (Fig. 4D). Oxygen consumption by cells reflects mitochondrial respiration and could be measured by oxygen consumption rate, namely OCR [20]. In the process of aerobic glycolysis, cells decrease the oxygen consumption rate. In consistence with the ECAR results, there was a significant increase in the OCR value after knockdown S100A4 expression (Fig. 4E), further reinforced the positive effect of S100A4 on aerobic glycolysis in CRC.

Glycolysis is a multistep enzymatic reaction involving multiple rate-limiting enzymes. To further understand the possible effects of S100A4 on glycolysis enzymes, we performed qRT-PCR to examine the transcriptional levels of these enzymes. As shown in Fig.4F, many metabolic enzyme genes have changed after silencing S100A4, with the most significance are the reduction of HK2, GLUT1 and LDHA. Collectively, these results suggested S100A4 as a positive regulator of glycolysis in CRC.

The uptake of ^{18}F-FDG by tumors is the reflection of the Warburg effect, and the PET/CT imaging system was developed on the theoretical basis of glycolysis, which was regarded as a powerful diagnostic method in cancer. All mice mentioned above underwent PET/CT scan before sacrificed. As anticipated, tumors derived from RKO-shS100A4 cells had significantly lower SUVmax value than tumors derived from control cells ($P < 0.05$; Fig. 4G and 4H). Taken together, these results confirmed that S100A4 is positive regulator of aerobic glycolysis in CRC.

S100A4 affected hypoxia-inducible factor-1α activity in CRC

Hypoxia-inducible factor (HIF)-1α is a core regulator of hypoxia, and is closely related to tumor growth and glycolysis in solid tumor. The stabilized HIF-1α could induce over-expression and increased activity of several glycolytic protein isoforms including transporters GLUT1, HK2, LDH-A, et al[21]. Therefore, S100A4 may promote CRC proliferation and aerobic glycolysis activity by enhancing HIF activity. HIF-1α acts by binding to the HIF-1α response element (HRE) on hypoxia. Therefore, we used HRE-luciferase reporter to determine whether S100A4 affected HRE-luciferase activities. As expected, S100A4 could increase HRE-luciferase activity in a dose-dependent manner (Fig. 5A). Moreover, HRE activity can be

significantly inhibited by silencing S100A4 expression using its specific shRNA (Fig. 5B), implying that S100A4 does affect the HIF-1α activity to promote proliferation and glycolysis in CRC.

Discussion

In this study, we demonstrated that ectopic expression of S100A4 was closely related to inferior prognosis factor, and S100A4 was validated as an independent prognostic factor for patients with CRC after colectomy in both univariate and multivariate survival analysis. Furthermore, S100A4 deficiency attenuated CRC cells proliferation and impaired glycolysis both in vivo and in vitro. Mechanistically, S100A4 activated proliferation and glycolysis in a HIF-1α-dependent manner.

S100A4, a member of the S100 calcium-binding protein family secreted by tumor and stromal cells, supports progress and metastasis in different ways. S100A4 promotes lung tumor development through inhibiting autophagy in a β-catenin signaling and S100A4 receptor RAGE-dependent manner[12]. It suppresses cancer stem cell proliferation via interaction with the IKK/NF-κB signaling pathway in bladder cancer[22]. In gastric cancer, S100A4-MYH9 axis promote migration and invasion of cancer cells by inducing TGF-β-mediated epithelial-mesenchymal transition[23]. Our results indicated that S100A4 expression in tumor tissues from CRC is closely linked to tumor progression, and this study is the first report in which S100A4 is shown to promote CRC growth by enhancing glycolysis.

The Warburg effect, a hallmark of cancer cells, has been highlighted in recent decades[24]. Coding and noncoding genes may regulate a number of metabolic enzymes, and the aberrantly expressed components might provide a growth advantage for cancer cells[5]. We got the conclusion that S100A4 was a positive regulator of proliferation and glycolysis from the following evidences. First, we performed a series of in vitro assays, including glucose uptake and lactate production assays and Seahorse assays to examine the correlation between S100A4 expression and glycolysis, and the results supported our hypothesis that S100A4 is a positive regulator of proliferation and glycolysis. Second, in vivo study, tumors derived from S100A4 knockdown cells were smaller and lighter than their controls provide further evidence that S100A4 promoted cell proliferation. Moreover, tumors from S100A4 cells showed lower SUVmax value than their controls, which provide firm evidences of our consumption.

HIF-1α plays an important role for cancer cell survival and metastases in the solid tumor under hypoxic microenvironment, in part by inducing glycolytic enzymes [25-27]. It can increase the glycolysis rate by up-regulating a series of glycolytic enzyme genes. When knockdown S100A4 expression, it could decreased several glycolytic enzymes expression that were also the transcriptiona target of HIF-1α. Hence, we hypothesis that S100A4 could up-regulate glycolysis activity by enhancing HIF-1α activity in CRC.

Luciferase study further confirmed that S100A4 could increase HRE activities at dose dependent. Thus, HIF-1α plays a central role in S100A4 mediated glycolysis in CRC.

Conclusion

Taken together, we uncovered a novel function of oncogene S100A4 in CRC progression and glycolysis regulation and discovered the possible molecular mechanism. These results identified S100A4 as a novel target for the diagnosis and treatment for CRC. Moreover, depriving the energy supply to control the tumor is a novel research direction that is actively being tried. S100A4 may provide a new potential target for such strategies.

Declarations

Availability of data and materials

All the datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

The study protocol and consent form were approved by the Institutional Ethics Committee of the Taizhou Municipal Hospital, Medical College of Taizhou University.

Consent for publication

All authors agreed to publish the article in the journal.

Competing interests

The authors declare that they have no competing interests

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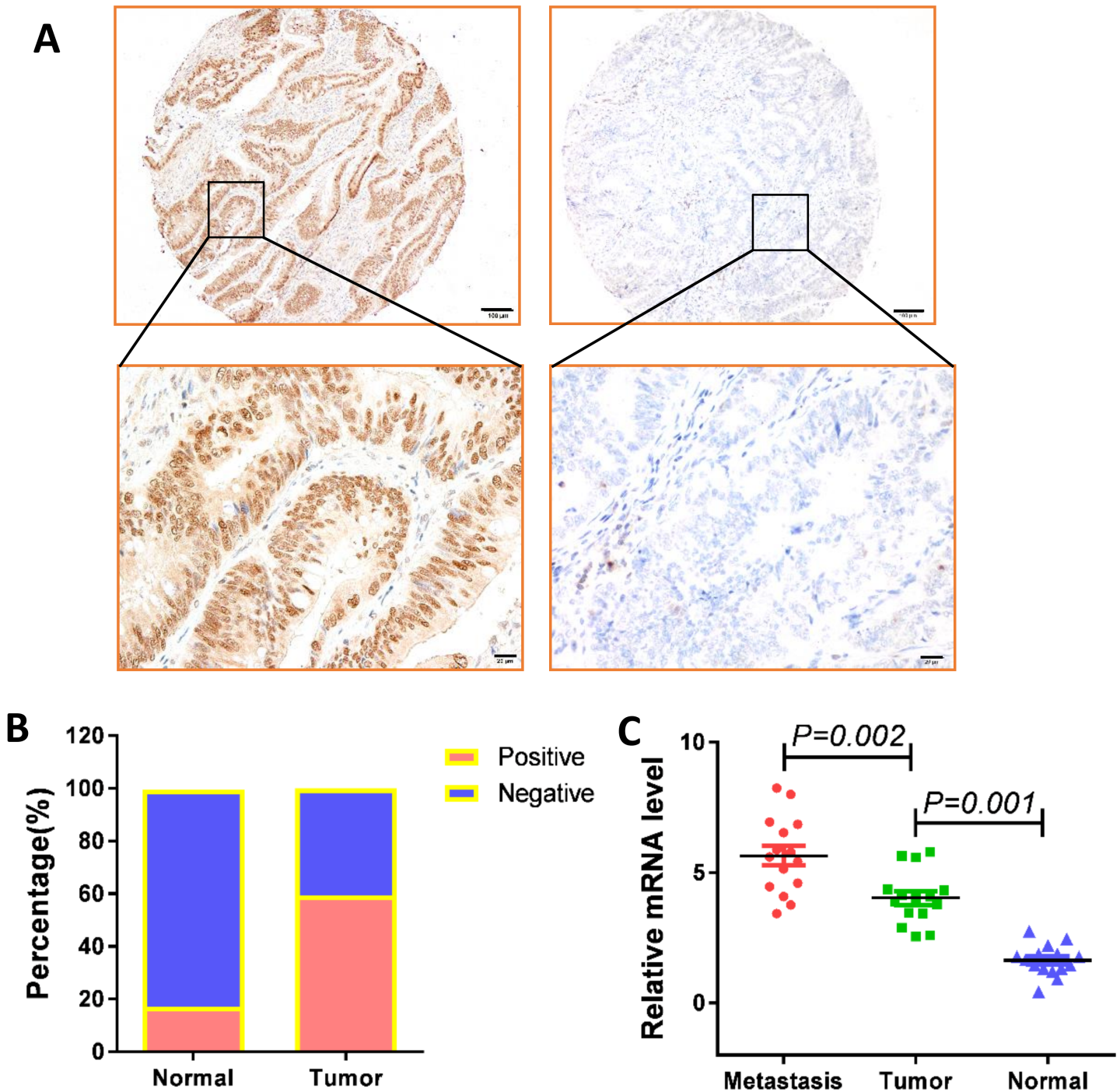
Figures

Figure 1

S100A4 expression patterns in CRC. (A) Representative images of S100A4 IHC staining in CRC. Amplified 100× in up panel, and 400× in down panel. (B) S100A4 expression was markedly higher than that in adjacent non-tumor tissues (59.4% vs 17.4%) (P<0.001). (C) Relative S100A4 mRNA levels in 15 matched liver metastases, primary tumors and adjacent normal mucosa specimens. The relative RQ value is used to represent the fold change in quantitative real-time polymerase chain reaction detection. S100A4 mRNA levels was found to be significantly different among the three groups (P< 0.001).
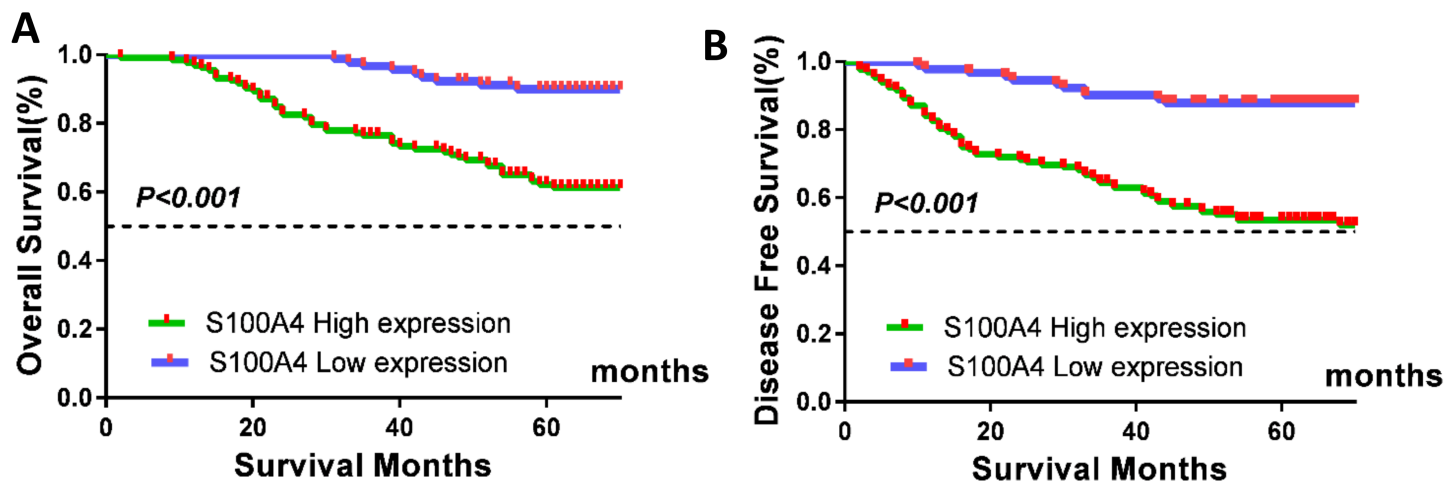
Figure 2

S100A4 is a predictor of survival in CRC. S100A4 expression was closely associated with patterns on overall survival (A) and disease free survival (B) by Kaplan-Meier analyses (P< 0.001).
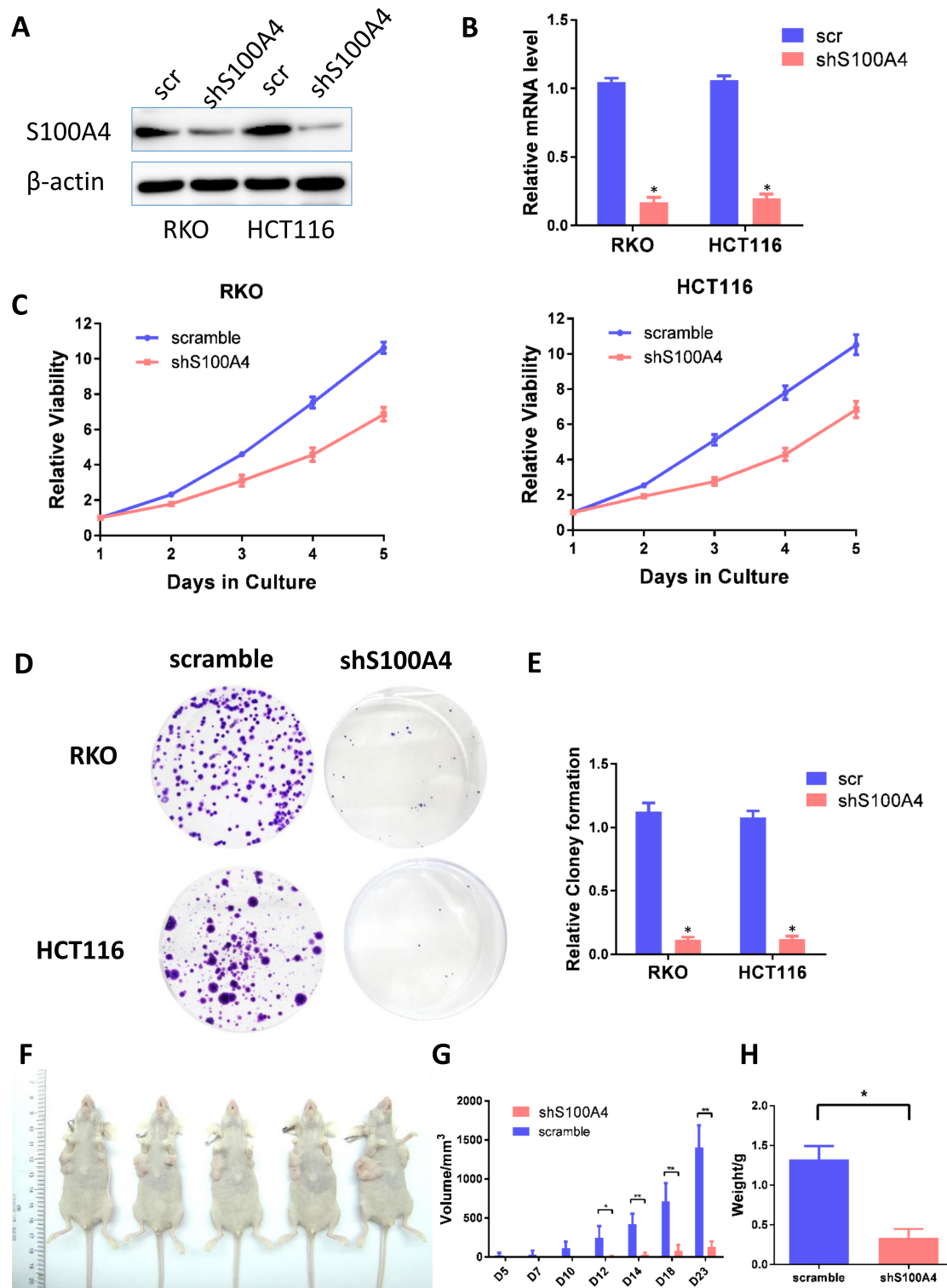
Figure 3

S100A4 associates with viability and proliferation ability of CRC cells. Efficiency of S100A4 knockdown in colon cancer cell lines was measured by Western blot analysis (A) and RT-PCR (B). Influence of S100A4 expression on viability of colon cancer cells was measured by CCK-8 assay (C) and clone formation (D-E). (E) Photographs of animals during the course of study. Colon cancer cells were paired subcutaneously injected into forelimbs in nude mice. The right sides were RKO-scramble and the left sides were RKO-

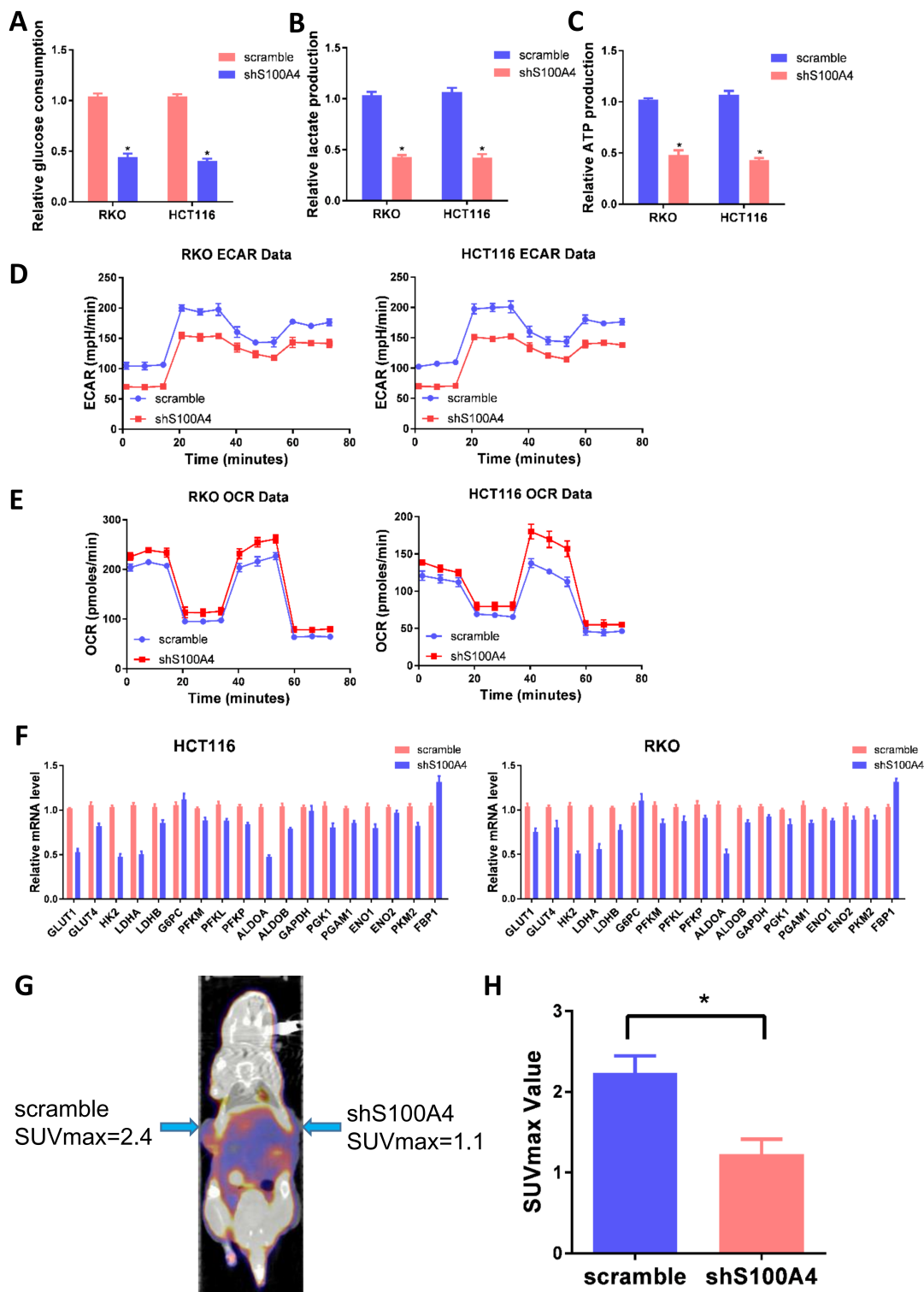shS100A4. Tumors volumes (G) and tumor weight (H) were measured on the indicated days (*P< 0.05, ** P< 0.001).

Figure 4

Effects of S100A4 on aerobic glycolysis in CRC. (A) Silencing S100A4 expression in RKO and HCT116 cells significantly decreased glucose utilization (A), lactate production (B), and ATP production(C). The impact of S100A4 on glycolysis rate was assessed by using Seahorse Energy Flux system through

examination of ECAR (D) and OCAR (E), which reflects the glycolytic and mitochondrial respiration, respectively. The result suggested that silencing S100A4 expression inhibited glycolysis. (F) Glycolysis is a multi-step enzymatic reaction involving with a series of rate-limiting enzymes, RT-PCR analysis indicated that silencing S100A4 expression impact series glycolytic enzymes with most significantly decreased in GLUT1, HK2 and LDHA. (G) Representative photographs of PET/CT of animals. All mice underwent evaluation with 18F-FDG PET/CT scan before sacrificed. The values of SUVmax were lower in RKO-scramble group than shS100A4 groups. *P < 0.05.
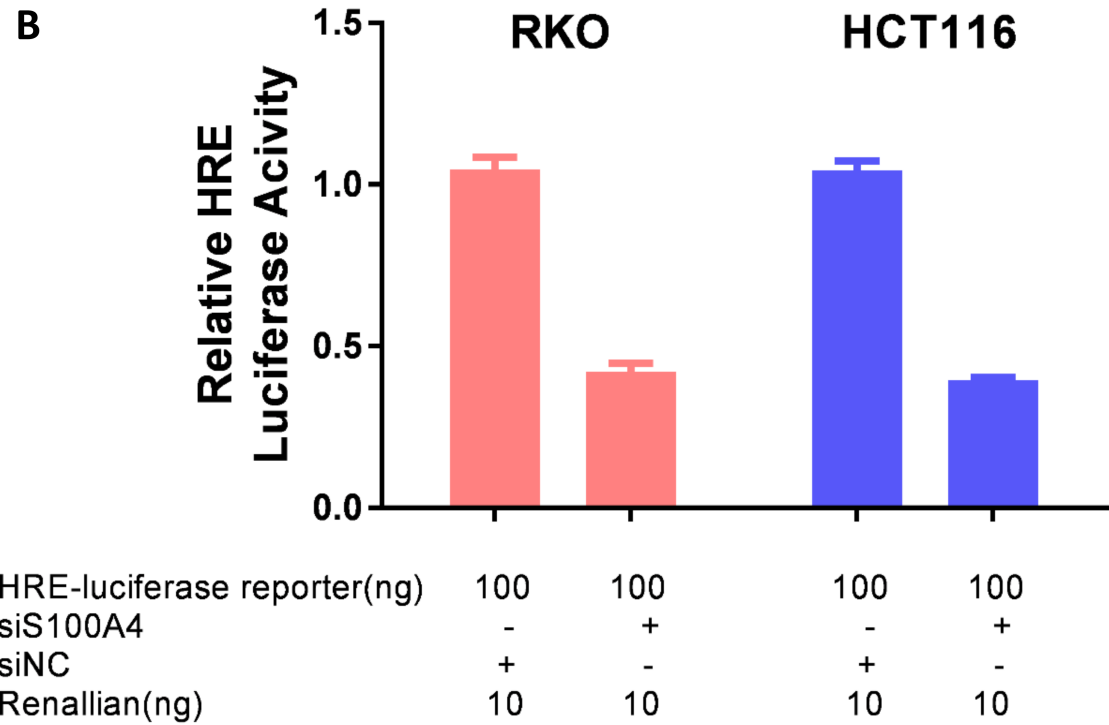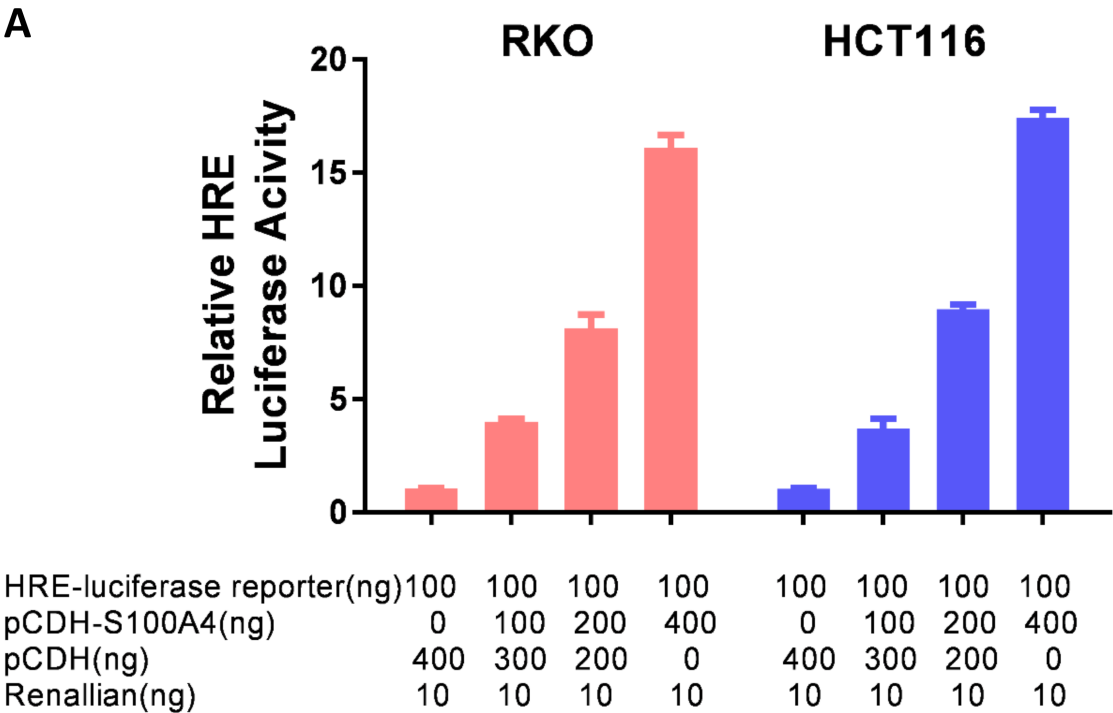
A

HRE-luciferase reporter(ng)	100	100	100	100	100	100	100	100
pCDH-S100A4(ng)	0	100	200	400	0	100	200	400
pCDH(ng)	400	300	200	0	400	300	200	0
Renallian(ng)	10	10	10	10	10	10	10	10

B

HRE-luciferase reporter(ng)	100	100	100	100
siS100A4	-	+	-	+
siNC	+	-	+	-
Renallian(ng)	10	10	10	10

Figure 5

S100A4 affected the HIF-1α activity in CRC cancer. (A) S100A4 could increase the HRE-luciferase activity in a dose-dependent manner (P < 0.05). (B) Silencing the expression of S100A4 decreased the HRE activity (P < 0.05).