Am J Transl Res 2022;14(8):5455-5465
www.ajtr.org /ISSN:1943-8141/AJTR0142768

Original Article

Down-regulation of NOTCH1 and PKM2 can inhibit the growth and metastasis of colorectal cancer cells

Jia Wang[1*], Meijuan Sun[2*], Rong Ma[3*], Gaobo Wang[1], Wenqing Li[1], Bowei Yang[1], Yang Yang[1]

[1]Department of General Surgery, Baoji City People's Hospital, Baoji 721000, Shaanxi, China; [2]Department of Pharmacy, Baoji Second People's Hospital, Baoji 721000, Shaanxi, China; [3]Department of Pathology, Baoji City People's Hospital, Baoji 721000, Shaanxi, China. [*]Equal contributors.

Received March 9, 2022; Accepted July 15, 2022; Epub August 15, 2022; Published August 30, 2022

Abstract: Background: Previous studies have revealed the overexpression of Notch receptor 1 (NOTCH1) and pyruvate kinase M2 (PKM2) in colorectal cancer (CRC) tissue and their relationship to disease development. However, whether there is synergy between PKM2 and NOTCH1 needs to be verified. This study aims to analyze the mechanism and relationship between NOTCH1 and PKM2 in CRC. Methods: Immunohistochemistry was used to measure the expression of NOTCH1 and PKM2 in colorectal cancer, and the correlation between them was analyzed by Pearson test. The protein and mRNA expressions in CRC cell lines were determined by western blot (WB) and real-time quantitative reverse transcription PCR (qRT-PCR). Compound 3K and tangeretin (TGN) were used to inhibit the expressions of PKM2 and NOTCH1, respectively. The wound healing assay and CCK-8 assay were applied to measure the migration and proliferation of cancer cells. Results: Immunohistochemical analysis showed that NOTCH1 and PKM2 were overexpressed in patients with colorectal cancer, and patients with overexpression showed a higher number of lymph node metastases and high tumor stage (III+IV) ($P<0.05$). In addition, Pearson test showed that the level of NOTCH1 was positively correlated with the level of PKM2 ($P<0.05$). WB and qRT-PCR showed that the protein and mRNA levels of NOTCH1 and PKM2 in colorectal cancer cells were significantly up-regulated ($P<0.05$). The inhibition of PKM2 and NOTCH1 had a synergistic effect on reducing the invasion and proliferation of CRC cells. Conclusion: NOTCH1 and PKM2 are highly expressed in CRC patients. Inhibiting the expression of NOTCH1 and PKM2 can inhibit the growth and metastasis of CRC cells, providing therapeutic targets for the treatment of CRC.

Keywords: NOTCH1, colorectal cancer, compound 3k, pyruvate kinase M2, tangeretin, synergistic impact

Introduction

Colorectal cancer (CRC) ranks third among the most common malignant tumors and fourth among primary causes of death from cancer worldwide [1]. The median lifespan of patients with metastatic or inoperable CRC under no therapy is approximate 8 months [2]. Since CRC is usually asymptomatic in the early phase, most patients have already entered the mid to late stage and missed the timing for surgery. Even if surgery is performed early, some patients will develop postoperative recurrence and metastasis. For these patients, chemotherapy is the primary treatment option. However, CRC cells (CRCCs) have strong resistance to many types of chemotherapeutic drugs. Accordingly, it is of profound importance to develop novel drugs for the chemotherapy of CRC.

Pyruvate kinase (PK), one crucial enzyme in glycolysis, releases energy while catalyzing phosphoenolpyruvate conversion into pyruvate. Mammalian cells produce 4 isoforms (PK: L, R, M1 and M2) [3]. PKM2 is found to have expression mainly in embryonic cells, actively proliferating tissue cells and adult stem cells. As the embryo develops, it is gradually replaced by several other isoenzymes. However, during tumorigenesis, PKM2 expression increases and gradually replaces other isozyme types [4]. Overexpressed PKM2 in CRC tissue indicates an unfavorable outcome [5]. Targeting PKM2 with compound 3K suppresses the development of CRC through inhibiting glycolysis [6].

The Notch signaling pathway is a strongly conserved intercellular signal transduction system that consists of the Notch ligand, Notch receptor and transcription factor CSL. The Notch

Table 1. Immunohistochemical staining scoring system

Ratio of positively stained cells	Score	Intensity	Score
<5%	0	Unstained	0
5%~25%	1	Faint yellow	1
26%~50%	2	Yellowish brown	2
51%~75%	3	Brown	3
>75%	4		

receptor, NOTCH1-4, is a transmembrane glycoprotein. NOTCH1 expression is closely related to unfavorable overall survival of CRC patients and, thus, is a possible prognostic biomarker [7].

This study was designed to investigate the association between PKM2 and the Notch signaling pathway, and determine whether simultaneous suppression of PKM2 and NOTCH1 with compound 3K and tangeretin (TGN), respectively, exert synergistic impacts against CRC. This method could provide a novel strategy for the therapy of CRC.

Materials and methods

Study approval and patient consent

The study was conducted with approval of the Ethics Committee of Lanzhou University Second Hospital (Lanzhou, CN, Ethics approval No. 2018010045), and informed consent form was signed by each patient prior to surgery and the study was in line with the ethical principles for medical research associated with human subjects in the *Declaration of Helsinki*.

Clinical data

CRC tissue samples (n=66) were collected from patients (age range 34-80 years old; mean age 60.3 years old) who underwent surgery in Lanzhou University Second Hospital from March 2018 to February 2019. The diagnosis of CRC was confirmed by an experienced pathologist. No patients had undergone chemotherapy or radiation therapy before resection. Inclusion criteria: Patients whose pathological stage met the diagnostic criteria of stage 8 CRC issued by American Joint Committee on Cancer in 2017; patients with complete clinical data; patients who were diagnosed with CRC for the first time. Exclusion criteria: Patients with immune diseases, cardiovascular or cerebrovascular diseases, intestinal obstruction or bleeding, and patients with other tumors.

Immunohistochemical analysis

The CRC tissue samples were immobilized, dehydrated and embedded in paraffin, then the samples were given treatment to obtain 4 μm-thick slices that were stained with hematoxylin. Prior to analysis, the tissue samples were treated by deparaffinization and rehydration through graded xylene and ethanol series, respectively. Sodium citrate was adopted for antigen retrieval, followed by sealing through H_2O_2. Afterwards, the sections were subjected to 16-h incubation (4°C) with primary antibodies against PKM2 and NOTCH1 and then 2-h incubation (indoor temperature) with horseradish peroxidase (HRP)-conjugated secondary antibody (Beijing Zhongshan Jinqiao Biological Technology Co., Ltd., Beijing, CN). The existence of the secondary antibody was detected by the Cell and Tissue Dyeing HRP-DAB Kit (Bei jing Zhongshan Jinqiao Biological Technology Co., Ltd.). An orthophoto microscope was used to obtain images of the stained tissue specimens.

All of the stained tissue samples were graded by two qualified pathologists using a two-level scoring method. The percentage of positive stained cancer cells of every section was counted, and the degree of staining was quantified. The grading system of the stained tissue specimens is shown in **Table 1**. The results of the two scores were multiplied, and a score <3 and a score of ≥3 implied negative protein expression and positive expression, respectively.

Cell incubation and drugs

Normal human colon epithelial NCM460 cells were offered by Hunan Fenghui Biotechnology Co., Ltd. (Hunan, CN), and human CRC RKO and HT29 cells were provided by Shanghai Institute of Cell Biology (CAS, Shanghai, CN). All cells were subjected to incubation (37°C) in 10% FBS (Gibco)-contained RPMI 1640 medium (Gibco, Carlsbad, CA, USA) under humidified environment (5% CO_2/95% air). Compound 3K was provided by Shanghai Selleck Chemicals Co., Ltd. (Wuhan, CN), and the NOTCH1 inhibitor TGN was purchased from MedChemExpress LLC (Monmouth Junction, NJ, USA). Antibo-

Table 2. Primer sequences

Gene	Upstream primer	Downstream primer
PKM2	5'-CAGAGGCTGCCATCTACCAC-3'	5'-GACGAGCTGTCTGGGGATTC-3'
NOTCH1	5'-TACAAGTGCGACTGTGACCC-3'	5'-ATACACGTGCCCTGGTTCAG-3'
β-actin	5'-TGGCACCCAGCACAATGAA-3'	5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'

dies against PKM2 were purchased from Affinity Biosciences (Cincinnati, OH, USA), those against NOTCH1 from Abcam (Cambridge, MA, USA), and those against GAPDH from Proteintech (Wuhan, CN).

CCK-8 assay

RKO and HT29 cells were transferred to 96-well plates (5000 cells/well) and treated with 1% dimethyl sulfoxide (DMSO) (as a control), compound 3K (9 μM for RKO cells and 6 μM for HT29 cells), TGN (25 μM), or the combination of compound 3K and TGN. After 24 h, every well was added with CCK-8 reagent (10 μL), and a microplate reader was adopted for measuring optical density (OD) at 450 nm. The cell proliferation activity (%) = (OD in the experimental group - that in CCK-8)/(that in the control group - that in CCK-8).

Wound healing assay

RKO and HT29 cells (5×10^5) were seeded in 6-well plates. Once the cells reached a confluence >95%, wounds were created via 200-μl and 10-μl sterile pipette tips for the RKO and HT29 cells, respectively. After removal of floating cells by phosphate-buffered saline and replacement of the medium, DMSO (as a control), compound 3K (9 μM for RKO cells and 6 μM for HT29 cells), TGN (25 μM), or a combination of compound 3K and TGN was added into the wells. The wound healing results during 24 h that could reveal migration activity were recorded under an optical microscope.

qRT-PCR

Total RNA was extracted from NCM460, RKO, and HT29 cells using TRIzol reagent (Takara Bio, Inc., Shiga, Japan) and treated by reverse transcription to obtain complementary DNA using a PrimeScript RT Reagent Kit (Takara Bio). The amplifications were conducted via SYBR Green Master Mix (Yeasen, Shanghai, CN) with gene-specific primers (internal control: β-actin). The primer sequences are summarized in **Table 2**.

Western blot assay

Total proteins were extracted from NCM460, RKO, and HT29 cells by homogenization in phenylmethylsulfonyl fluoride-contained radioimmunoprecipitation assay. Equivalent amounts of proteins (30 μg/lane) were isolated through electrophoresis with 10% sodium dodecyl sulfate gels, followed by transfer onto PVDF membranes that were treated with 2-h immersion (indoor temperature) in 5% defatted milk in TBST and then incubation (at 4°C) all night with primary antibodies against NOTCH1 (1:1000), PKM2 (1:1000) and GAPDH (1:5000). Subsequently, the membranes were cleaned with TBST, followed by culture with HRP-conjugated goat anti-rabbit IgG secondary antibodies. ChemiDoc™ Gel Imaging System (Bio-Rad Laboratories, Hercules, CA, USA) was adopted for visualizing protein bands.

Statistical analyses

All data were analyzed using the Graphprism Pad 8 (graphics board software company, San Diego, California, USA) and SPSS 26.0 (SPSS Company, Chicago, Illinois, USA). The measured data were expressed as mean ± standard deviation. Comparison between the two groups was by independent sample t-test. One way ANOVA was used for multigroup comparison, and LSD-t test for post test. The repeated measurement ANOVA and post test at different time points were performed by Bonferroni test. Pearson test was used to analyze the correlation between genes. The counted data were expressed in percentage (%) and compared by Chi square test. Cox regression analysis was used to analyze the prognostic factors of patients. A difference of $P<0.05$ was considered significant.

Results

Upregulation of NOTCH1 and PKM2 in CRC tissue

Overexpression of PKM2 and NOTCH1 were found in 71.2% (47/66) and 68.2% (45/66) of

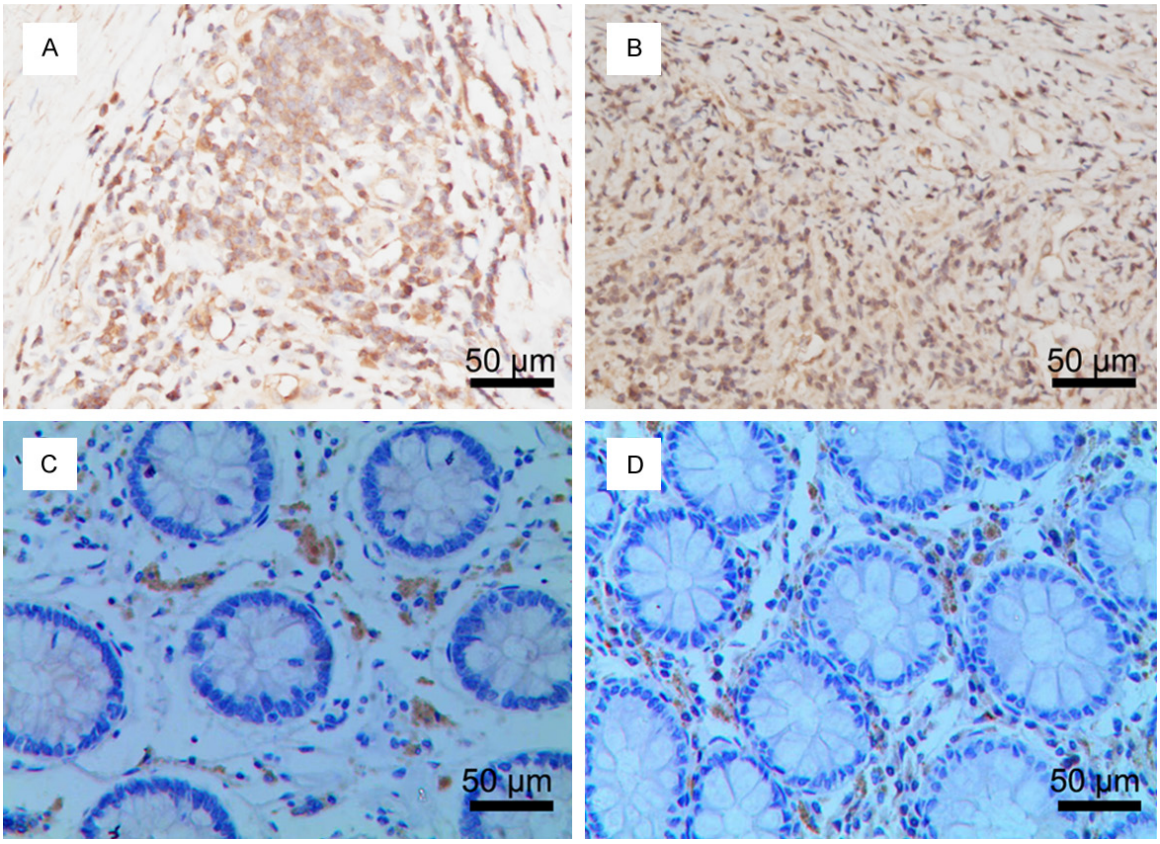

Figure 1. Immunohistochemistry. (A and B) show positive expression of PKM2 and NOTCH1 in colorectal cancer tissue, while (C and D) show no expression of the two in normal tissue (×200).

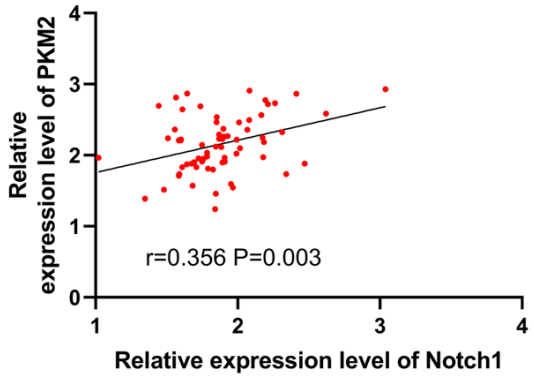

Figure 2. Analysis of correlation between PKM2 and NOTCH1 in colorectal cancer tissue.

the CRC samples, respectively (**Figure 1**), and clear co-expression was also revealed (**Figure 2**, R=0.356, *P*<0.01). Notably, there were significant correlations of PKM2 with lymph node metastasis (LNM), sex, age and tumor stage, and of NOTCH1 with tumor stage and LNM (**Table 3**, P<0.05). The patients were followed up for 3 years. Through multivariate Cox regression analysis, it was found that NOTCH1 and PKM2 were independent risk factors affecting prognosis (**Table 4**, P<0.05)

Upregulation of PKM2 and NOTCH1 in human CRCCs

In human CRC RKO and HT29 cells and human normal colorectal epithelial cell NCM460 cells, the protein and mRNA levels of PKM2 and NOTCH1 were evaluated by WB/qRT-PCR assay. The results demonstrated significant upregulation in the mRNA (**Figure 3A**, **3B**) and protein (**Figure 3C-F**) levels of PKM2 and NOTCH1 in CRCCs.

Influences of PKM2 and NOTCH1 on mobility and proliferation of CRCCs

According to CCK-8 assay, suppressing PKM2 or NOTCH1 significantly reduced the proliferation of RKO and HT29 cells (**Figure 4A**, **4B**, *P*<0.01). The combination of compound 3K and TGN greatly suppressed the proliferation of RKO and HT29 cells compared with either individually and DMSO at 24 h. According to the results of wound healing assay, compound 3K

Table 3. Relationship between PKM2 and NOTCH1 expressions and clinical features of colorectal cancer

Clinical characteristic		n	PKM2		*P* value	NOTCH1		*P* value
			-	+		-	+	
Sex					0.012			0.491
Male		40	7	33		14	26	
Female		26	12	14		7	19	
Age (years)					0.016			0.324
<60		31	4	7		8	23	
≥60		35	15	20		13	22	
Tumor stage					0.01			0.041
I-II		35	13	22		15	20	
III-IV		31	6	25		6	25	
LNM					0.047			0.028
No		36	14	22		15	21	
Yes		30	5	25		5	25	
Tumor marker								
CEA	<5	44	14	30	0.58	12	32	0.309
	≥5	20	5	15		8	12	
CA199	<27	45	13	32	0.83	13	32	0.531
	≥27	19	6	3		7	12	
CA125	<40	54	16	38	0.981	18	36	0.642
	≥40	10	3	7		2	8	

Table 4. Cox regression analysis

Clinical characteristic	Univariate Cox			Multivariate Cox		
	P value	HR value	95 CI%	*P* value	HR value	95 CI%
Sex	0.517	0.812	0.433-1.523			
Age (years)	0.410	0.769	0.412-1.436			
Tumor stage	0.614	1.173	0.630-2.185			
LNM	0.753	1.105	0.593-2.063			
CEA	0.633	1.172	0.611-2.246			
CA199	0.718	1.127	0.588-2.160			
CA125	0.155	1.717	0.816-3.614			
PKM2	0.010	3.130	1.310-7.477	0.029	2.645	1.102-6.348
NOTCH1	0.003	3.816	1.595-9.129	0.007	3.350	1.394-8.050

+ TGN notably suppressed the migration of both RKO and HT29 cells compared to either individually and to DMSO (**Figure 5A-C**, $P<0.01$). Collectively, the results revealed that compound 3K and TGN exerted synergistic impacts on cell mobility and proliferation.

Relationship between PKM2 and NOTCH1 in CRCCs

The results revealed that suppression of PKM2 decreased the protein/mRNA levels of NOTCH1 in RKO and HT29 cells, while the combination of compound 3K and TGN exerted stronger inhibitory impacts in contrast to either individually. Interestingly, suppression of NOTCH1 decreased PKM2 in both protein and mRNA levels, and the combination of compound 3K and TGN exerted stronger inhibitory impacts in contrast to the single drugs. It follows from these results that compound 3K and TGN exert a synergistic impact on expressions of PKM2 and NOTCH1 (**Figure 6**).

Discussion

Colorectal cancer (CRC) has a high incidence and is a main causes of cancer-associated

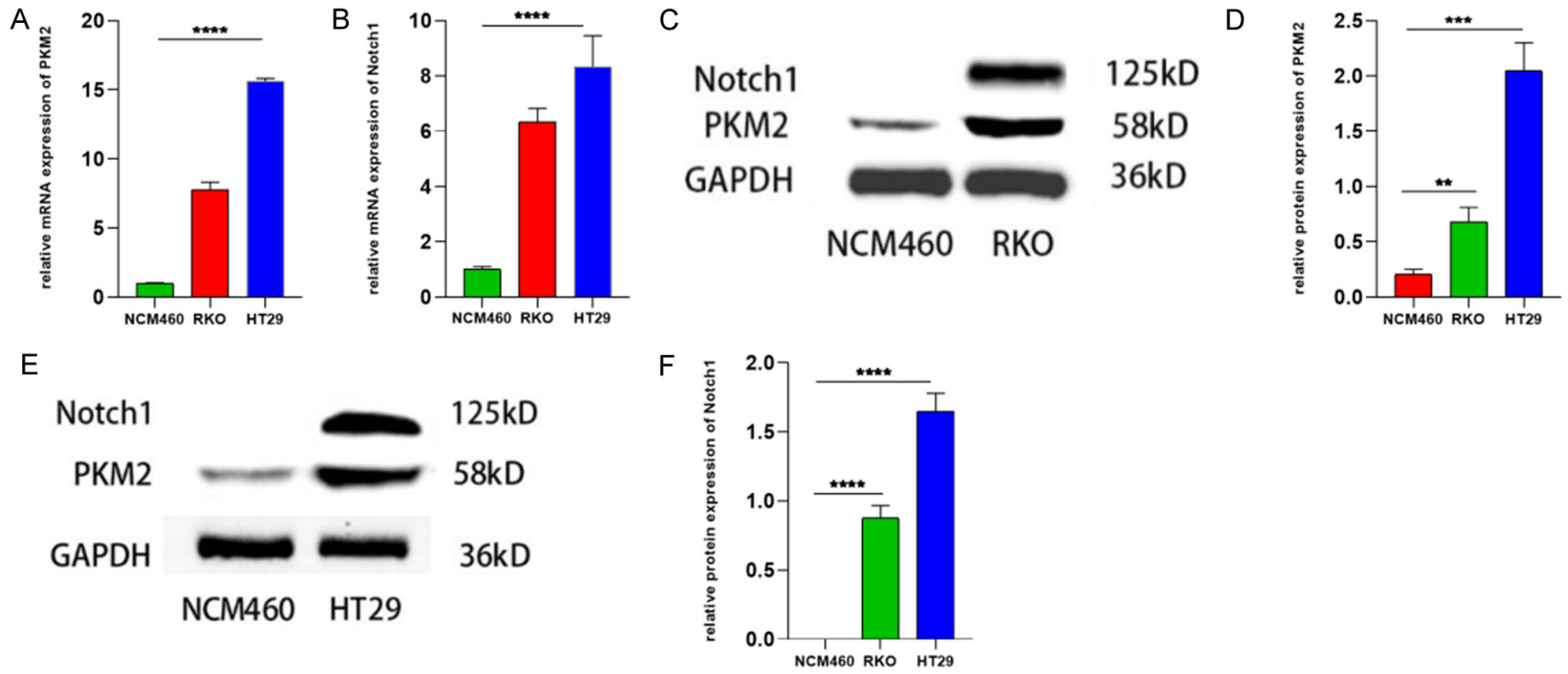

Figure 3. NOTCH1 and PKM2 expressions in colorectal cancer cells. A, B. qRT-PCR was utilized to determine mRNA levels of PKM2 and NOTCH1 in colorectal cancer cells. C-F. Western blot was utilized to determine the protein levels of PKM2 and NOTCH1 in colorectal cancer cells. ***P<0.001; **P<0.01; ****P<0.0001.

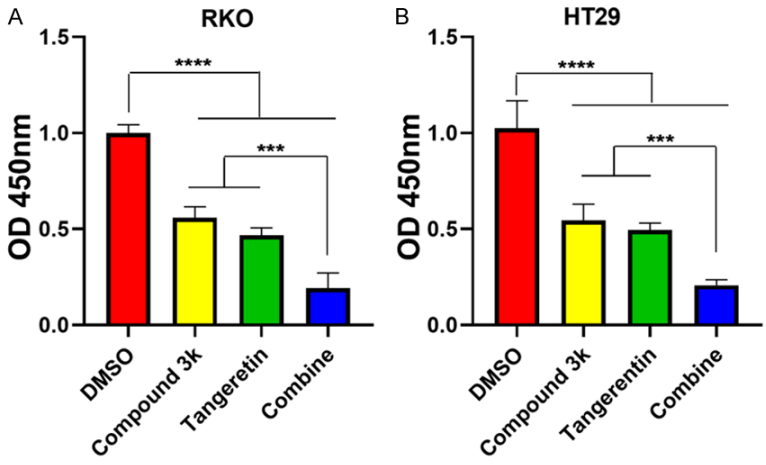

Figure 4. Compounds 3K and TGN exerted synergistic impacts on the proliferation of RKO and HT29 cells. A, B. CCK-8 test was used to evaluate the proliferation of colorectal cancer cells after 24-h incubation with DMSO, compound 3K, TGN, or the combination of compounds 3K and TGN. ****P<0.0001, ***P<0.001.

mortality [8, 9]. Accordingly, we need to study its carcinogenesis mechanisms to devise therapys.

PKM2, with high expression in various types of cancers such as CRC, acts as a catalyst for the final link of glycolysis and readjusts glycolytic flux to meet the specific cell metabolic requirements for proliferation [10-13]. The Notch signaling pathway is critical for understanding cell fate in multicellular organisms [14]. Abnormal expression of NOTCH1 has been attributed to CRC severity [15], and high NOTCH1 protein expression is linked to unfavorable outcome in CRC [16].

In this study, we revealed the high protein coexpression of NOTCH1 and PKM2 in CRC tissue by immunohistochemical analysis. In addition, compared to NCM460 cells, the protein and mRNA levels of PKM2 and NOTCH1 were highly expressed in RKO and HT29 cells. In the studies of Chu [17] and Cui [18], NOTCH1 and PKM2 were overexpressed in patients with CRC, and their high expressions were related to poor prognosis. The above results are basically consistent with the present study, indicating that PKM2 and NOTCH1 are related to the development of CRC, and targeting PKM2 or NOTCH1 through gene silencing or pharmacological method is an effective anticancer strategy. Compound 3K is another PKM2 inhibitor, which has an antiproliferative effect on liver and colon cancer [19]. TGN is a specific inhibitor of NOTCH1, which is effective on gastric cancer cells [20]. In order to further explore the mechanism of PKM2 and NOTCH1 in CRC, we used inhibitors to observe the effects of inhibition of PKM2 and NOTCH1 on CRCCs.

In our study, compounds 3K and TGN were adopted for understanding the feasibility of simultaneous suppression of NOTCH1 and PKM2 in CRCCs. According to the results, suppression of PKM2 greatly downregulated NOTCH1 expression in addition to the mobility and proliferation of CRCCs. Interestingly, inhibition of NOTCH1 had a notable effect on PKM2 expression and on the mobility and proliferation of CRCCs. Therefore, the focus of this study was the relationship between PKM2 and NOTCH1. This study showed for the first time that PKM2 and NOTCH1 were positively correlated. Inhibition of PKM2 and NOTCH1 could reduce the proliferation and metastasis of CRC. In addition, PKM2 and NOTCH1 exerted synergistic inhibitory impacts on the mobility and proliferation of CRCCs. Collectively, it follows from these results that simultaneous suppression of NOTCH1 and PKM2 provides a strong strategy for the therapy of CRC.

In this study, we determined that PKM2 and NOTCH1 were highly expressed in CRC, and CRCC proliferation and metastasis could be reduced by inhibiting PKM2 and NOTCH1. However, this study still has some limitations. In this study, we conducted only a 3-year instead of a long-term follow-up in the patients. It is still unclear whether PKM2 and NOTCH1 are related to long-term survival. Secondly, in this study, we detected only the changes in cell proliferation and migration. Whether there is an impact on apoptosis and invasion needs to be further studied. Finally, no *in vivo* study was conducted, and whether the same effect is observable *in vivo* needs to be verified. Therefore, we hope to carry out prospective research in the future and conduct animal model experiments to improve our research conclusions.

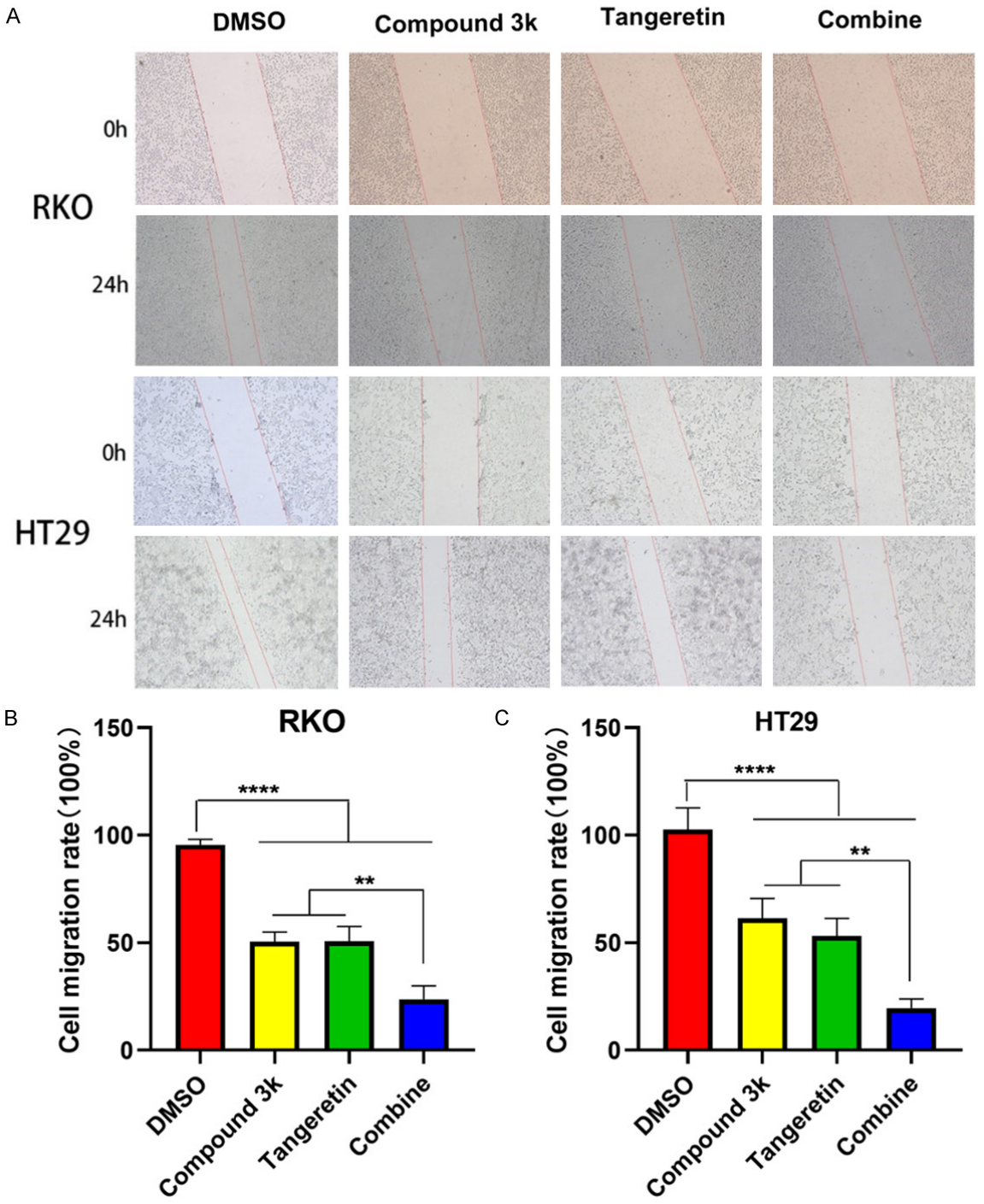

Figure 5. Compound 3K and TGN exerted synergistic impacts on the mobility of RKO and HT29 cells. A-C. The wound healing assay was carried out for assessing the migration of colorectal cancer cells after 24-h incubation with DMSO (as a control), compound 3K, TGN, or the combination of compounds 3K and TGN. ***P*<0.01; *****P*<0.0001.

Conclusion

PKM2 and NOTCH1 form one positive feedback loop that promotes CRC development. Compound 3K and TGN exert synergistic impacts against the mobility and proliferation of CRCCs. Therefore, simultaneous inhibition of PKM2 and NOTCH1 with compounds 3K and TGN, respectively, provides a novel and strong strategy for the therapy of CRC.

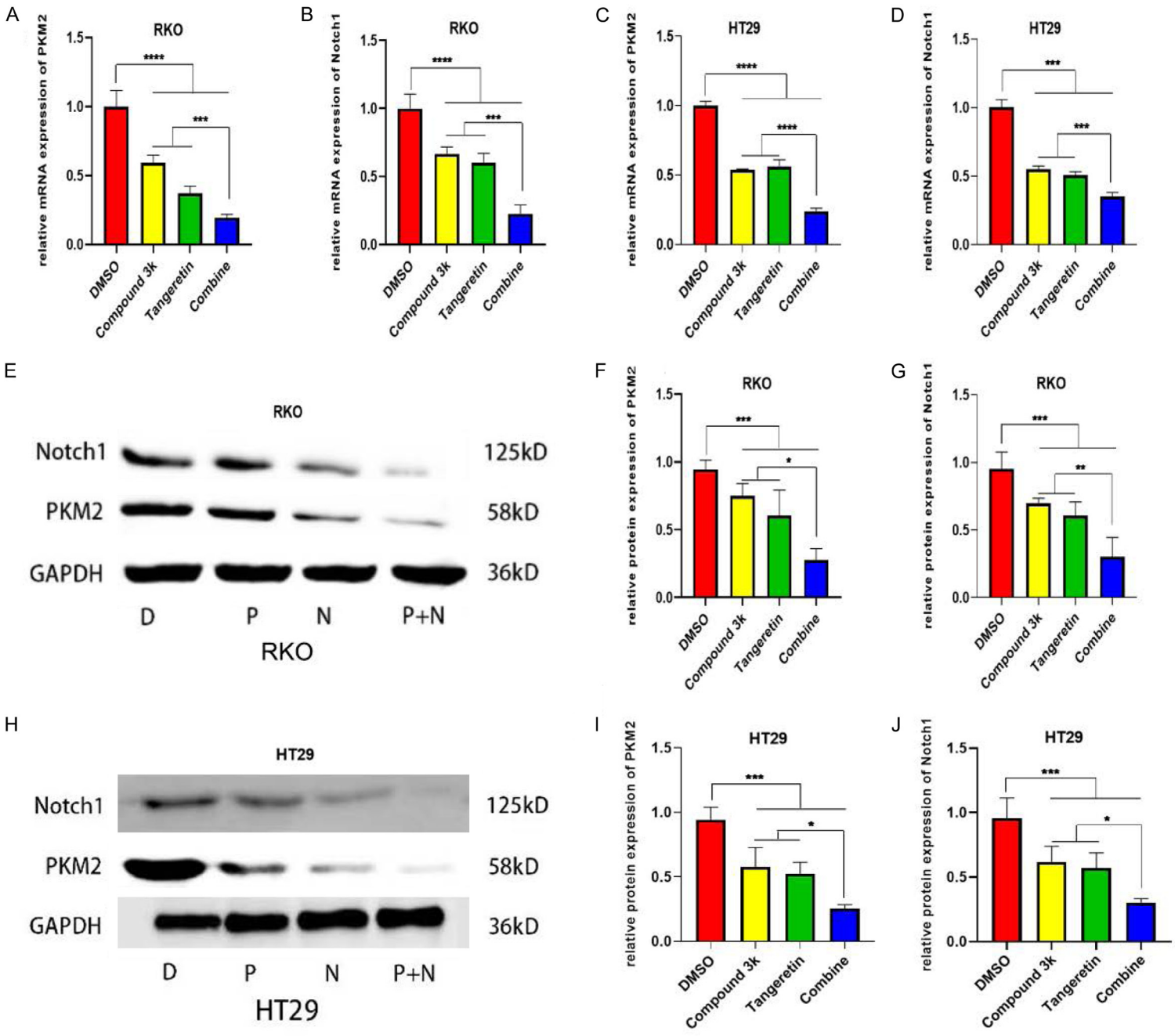
A
RKO
relative mRNA expression of PKM2
B
RKO
relative mRNA expression of Notch1
C
HT29
relative mRNA expression of PKM2
D
HT29
relative mRNA expression of Notch1
DMSO
Compound 3k
Tangeretin
Combine
E
RKO
Notch1
PKM2
GAPDH
125kD
58kD
36kD
D
P
N
P+N
RKO
F
RKO
relative protein expression of PKM2
G
RKO
relative protein expression of Notch1
H
HT29
Notch1
PKM2
GAPDH
125kD
58kD
36kD
D
P
N
P+N
HT29
I
HT29
relative protein expression of PKM2
J
HT29
relative protein expression of Notch1

Figure 6. PKM2 and NOTCH1 expressions in colorectal cancer cells treated with compounds 3K and TGN. A-D. qRT-PCR was carried out for measuring mRNA levels of PKM2 and NOTCH1 in the 4 groups of colorectal cancer cells after 24-h incubation with DMSO (as a control), compounds 3K, TGN, or the combination of compound 3K and TGN. E-J. Western blot was conducted to determine the protein levels of PKM2 and NOTCH1 in the 4 groups after 24 h incubation. *P<0.05; **P<0.01; ***P<0.001, D, DMSO; P, Compound 3K; N, tangeretin; P+N, Compound 3K+tangeretin.

Disclosure of conflict of interest

None.

Address correspondence to: Yang Yang, Department of General Surgery, Baoji City People's Hospital, Jinger Road Xinhua Lane 24, Baoji 721000, Shaanxi, China. Tel: +86-0917-3272900; E-mail: yangyang_baoji@163.com

References

[1] Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut 2017; 66: 683-691.

[2] Attoub S, Arafat K, Khalaf T, Sulaiman S and Iratni R. Frondoside A enhances the anti-cancer effects of oxaliplatin and 5-fluorouracil on colon cancer cells. Nutrients 2018; 10: 560.

[3] Chen J, Xie J, Jiang Z, Wang B, Wang Y and Hu X. Shikonin and its analogs inhibit cancer cell glycolysis by targeting tumor pyruvate kinase-M2. Oncogene 2011; 30: 4297-306.

[4] Hacker HJ, Steinberg P and Bannasch P. Pyruvate kinase isoenzyme shift from L-type to M2-type is a late event in hepatocarcinogenesis induced in rats by a choline-deficient/DL-ethionine-supplemented diet. Carcinogenesis 1998; 19: 99-107.

[5] Cui R and Shi X. Expression of pyruvate kinase M2 in human colorectal cancer and its prognostic value. Int J Clin Exp Pathol 2015; 8: 11393-9.

[6] Ning X, Qi H, Li R, Jin Y, McNutt MA and Yin Y. Synthesis and antitumor activity of novel 2, 3-didithiocarbamate substituted naphthoquinones as inhibitors of pyruvate kinase M2 isoform. J Enzyme Inhib Med Chem 2018; 33: 126-129.

[7] Chu D, Li Y, Wang W, Zhao Q, Li J, Lu Y, Li M, Dong G, Zhang H, Xie H and Ji G. High level of NOTCH1 protein is associated with poor overall survival in colorectal cancer. Ann Surg Oncol 2010; 17: 1337-42.

[8] Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi RE and Corcione F. Worldwide burden of colorectal cancer: a review. Updates Surg 2016; 68: 7-11.

[9] Szarvas T, Csizmarik A, Nagy N, Keresztes D, Váradi M, Küronya Z, Riesz P and Nyirády P. Molecular underpinnings of systemic treatment resistance in metastatic castration-resistant prostate cancer. Orv Hetil 2020; 161: 813-820.

[10] Iqbal M, Gupta V, Gopinath P, Mazurek S and Bamezai RN. Pyruvate kinase M2 and cancer: an updated assessment. FEBS Lett 2014; 588: 2685-92.

[11] Shiroki T, Yokoyama M, Tanuma N, Maejima R, Tamai K, Yamaguchi K, Oikawa T, Noguchi T, Miura K, Fujiya T, Shima H, Sato I, Murata-Kamiya N, Hatakeyama M, Iijima K, Shimosegawa T and Satoh K. Enhanced expression of the M2 isoform of pyruvate kinase is involved in gastric cancer development by regulating cancer-specific metabolism. Cancer Sci 2017; 108: 931-940.

[12] Wang C, Jiang J, Ji J, Cai Q, Chen X, Yu Y, Zhu Z and Zhang J. PKM2 promotes cell migration and inhibits autophagy by mediating PI3K/AKT activation and contributes to the malignant development of gastric cancer. Sci Rep 2017; 7: 2886.

[13] Yasumizu Y, Hongo H, Kosaka T, Mikami S, Nishimoto K, Kikuchi E and Oya M. PKM2 under hypoxic environment causes resistance to mTOR inhibitor in human castration resistant prostate cancer. Oncotarget 2018; 9: 27698-27707.

[14] Ulvklo C, Macdonald R, Bivik C, Baumgardt M, Karlsson D and Thor S. Control of neuronal cell fate and number by integration of distinct daughter cell proliferation modes with temporal progression. Development 2012; 139: 678-89.

[15] Tyagi A, Sharma A and Damodaran C. A review on notch signaling and colorectal cancer. Cells 2020; 9: 1549.

[16] Arcaroli JJ, Tai WM, Mcwilliams R, Bagby S, Blatchford PJ, Varella-Garcia M, Purkey A, Quackenbush KS, Song EK, Pitts TM, Gao D, Lieu C, McManus M, Tan AC, Zheng X, Zhang Q, Ozeck M, Olson P, Jiang ZQ, Kopetz S, Jimeno A, Keysar S, Eckhardt G and Messersmith WA. A NOTCH1 gene copy number gain is a prognostic indicator of worse survival and a predictive biomarker to a NOTCH1 targeting antibody in colorectal cancer. Int J Cancer 2016; 138: 195-205.

[17] Chu D, Zhou Y, Zhang Z, Li Y, Li J, Zheng J, Zhang H, Zhao Q, Wang W, Wang R and Ji G.

NOTCH1 expression, which is related to p65 Status, is an independent predictor of prognosis in colorectal cancer. Clin Cancer Res 2011; 17: 5686-5694.

[18] Cui R and Shi XY. Expression of pyruvate kinase M2 in human colorectal cancer and its prognostic value. Int J Clin Exp Pathol 2015; 8: 11393-11399.

[19] Zeng Z, Lan J, Lei S, Yang Y, He Z, Xue Y and Chen T. Simultaneous inhibition of ornithine decarboxylase 1 and pyruvate kinase m2 exerts synergistic effects against hepatocellular carcinoma cells. Onco Targets Ther 2020; 13: 11697-11709.

[20] Zhang X, Zheng L, Sun Y, Wang T and Wang B. Tangeretin enhances radiosensitivity and inhibits the radiation-induced epithelial-mesenchymal transition of gastric cancer cells. Oncol Rep 2015; 34: 302-10.