The relationship between *RECK* Gene Polymorphisms and the prognosis of cholangiocarcinoma

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Abstract

Background

The study was designed to examine the reversion inducing cysteine rich protein with Kazal motifs (*RECK*) levels in patients with cholangiocarcinoma (CCA) and assess its role in CCA prognosis.

Methods

Quantitative real-time PCR (qRT-PCR) was used to determine the expression of *RECK* mRNA in 127 pairs of CCA samples and controls. Chi-square test was conducted to analyze the effects of clinical features on *RECK* expression. Kaplan-Meier curves were plotted to determine the overall survival rate of CCA patients with different *RECK* expression. The prognostic biomarkers for CCA patients were identified using the Cox regression analysis.

Results

Significantly down-regulated expression of *RECK* mRNA was determined in CCA tissues compared to noncancerous controls ($P < 0.05$). Chi-square test suggested reduced *RECK* expression was related with invasion depth ($P = 0.026$), differentiation ($P = 0.025$), lymphatic metastasis ($P = 0.010$) and TNM stage ($P = 0.015$). However, age, sex, tumor size and family history had no significant links with *RECK* expression (all, $P > 0.05$). The survival curves showed that patients with low *RECK* expression had a shorter overall survival rate than those with high *RECK* expression. Both the univariate analysis ($P = 0.000$, HR = 5.290, 95%CI = 3.195–8.758) and multivariate analysis ($P = 0.000$, HR = 5.376, 95%CI = 2.231–8.946) demonstrated that *RECK* was an independent biomarker for predicting the outcomes of CCA patients.

Conclusions

Taken together, the expression of *RECK* was down-regulated in CCA and it might be an efficient biomarker for CCA patients.

Background

Cholangiocarcinoma (CCA) is one of the most frequent cancers in the biliary tract with high malignancy. It constitutes approximately 2% of all reported cancers anfd accounts for about 3% of all gastrointestinal malignancies [1, 2]. According to the tumor location, CCA can be anatomically divided into two large subtypes: extrahepatic cholangiocarcinoma (EHCC) and intrahepatic cholangiocarcinoma (IHCC), and the former is further classified as perihilar CCA and distal CCA [3, 4] The mortality and morbidity of CCA have steadily increased in recent years all over the world [5]. At present, there are several treatment approaches for CCA patients, such as endoscopic stent placement, radiation therapy, surgical resection,

chemotherapy and photodynamic therapy [6]. Surgery is the only curative method for patients with early stage, however, due to late diagnosis and metastasis, the resection rate of CCA patients is always low and the recurrent rate is sometimes high [7, 8]. Therefore, the prognosis of CCA patients is extremely poor, which is often less than 40% in general [9, 10]. Thus, it is urgent to find novel biomarkers to predict the outcomes of patients with this disease and improve the treatment results.

Tumor invasion and metastasis of malignancies, which are signaled by the degradation of extracellular matrix (ECM), are the important factors for cancer-related deaths [11, 12]. Matrix metalloproteinases (MMPs) and their inhibitors have been reported to play important roles in the ECM degradation [13, 14]. *RECK* (reversion inducing cysteine rich protein with Kazal motifs) is a newly found tumor suppressor gene, which could inhibit the expression and activity of MMPs as an inhibitor [15, 16]. The RECK protein is a 110-kDa glycosyphosphatidylinositol (GPI)-anchored protein in mammal cells, which is involved in a variety of biological processes, such as tissue remodeling, remodeling enzyme control and vascular grow during the development [17, 18]. Reduced expression of *RECK* has been found in various cancers, including breast cancer, bladder carcinoma and prostate cancer [19-21].

In the present study, we attempted to measure the expression of *RECK* in CCA patients and then to identify its prognostic role in CCA.

Methods

Patients and specimens

A total of 127 CCA patients who received surgical resection in the PLA Rocket Force Characteristic Medical Center were enrolled in this retrospective study, including 67 male cases and 60 female cases. None patients were subjected to radio- or chemo- therapy before surgery. The resected tissue samples, including 127 CCA specimens and 127 adjacent noncancerous controls were collected immediately after surgery and stored at -80℃ until use. A 5-year follow-up was taken and the clinical information of patients was updated every 3 months through telephone calls. This study was approved by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center. The informed consents were provided by the patients before surgery.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from CCA and control tissues with the RNeasy Mini Kit (Qiagen, Germantown) according to the manufacture's instructions. The SuperScript® III Reverse Transcriptase Kit (Life Technologies, Carlsbad, CA, USA) was adopted for cDNA synthesis. Quantitative real time PCR was performed with QuantiTect SYBR Green RT-PCR Kit (Qiagen). The gene *GAPDH* was used as an endogenous control. The relative expression levels of *RECK* were calculated by the $2^{-\Delta\Delta Ct}$ method. All experiments were performed in triple.

Statistical analysis

All data were statistically analyzed on SPSS 18.0 and Sigmaplot 12.5 softwares. The relationships of *RECK* expression with various clinical characteristics were detected by Chi-square test. The overall survival rate was evaluated by the Kaplan-Meier analysis and log-rank test . Cox regression analysis was applied to identify the prognostic factors for CCA prognosis. All data were presented as mean±SD. The results were significant with *P* values of less than 0.05.

Results

Down-regulation of *RECK* mRNA in CCA tissues

The expression of *RECK* mRNA was measured using qRT-PCR. As shown in **Figure 1**, the relative expression level of *RECK* mRNA in CCA tissues was 1.705±0.476 (mean±SD), while that in adjacent normal controls was 3.188±0.431. Thus the expression of *RECK* in CCA was significantly lower than that in controls ($P<0.05$).

Association between *RECK* expression and clinical characteristics of CCA patients

To explore the relationship between *RECK* expression and clinical characteristics of CCA patients, Chi-square test was conducted. Significant correlations were observed between *RECK* expression and invasion depth ($P=0.026$, differentiation ($P=0.025$), lymphatic metastasis ($P=0.010$) and TNM stage ($P=0.015$). However, no significant relation was observed between *RECK* levels and age, sex, tumor size or family history (all, $P>0.05$) (**Table 1**).

Low-expression of *RECK* was associated with poor prognosis in CCA patients

Among all the 127 CCA patients, 75 (59.05%) cases died of this disease during the 5-year follow-up, with the 5-year overall survival rate of 40.95%. As shown in **Figure 2**, the survival rate was 18.75% in low-*RECK* group, and 63.49% in high-*RECK* group ($P<0.001$). Univariate analysis demonstrated that invasion depth ($P=0.021$), differentiation ($P=0.042$), TNM stage ($P=0.004$) and *RECK* level ($P=0.000$) were related with prognosis of CCA patients (**Table 2**). Meanwhile, the multivariate analysis further revealed that TNM stage ($P=0.004$, HR=1.972, 95%CI=1.242-3.131) and *RECK* level ($P=0.000$, HR=5.376, 95%CI=3.231-8.946) were independent biomarkers for CCA prognosis.

Discussion

CCA refers to those malignancies that originate from the epithelial cells of the bile duct system, and is only inferior to liver cancer. CCA is characterized by jaundice, stomachache, eallbladder enlargement, fever, and ascites. There are no specific clinical manifestations for CCA at early stages, resulting in only a small part of patients could receive the curative resection. The 5-year survival rate of CCA patients is low and the the prognosis is dismal. Thus, new predictor biomarkers are in urgent need to improve the prognosis of CCA patients. So far, some prognostic markers have been identified for CCA patients. For instance, Wu et al. claimed that *S100P* was a candidate for prognosis, diagnosis and treatment of CCA [22]. In the study of Gu et al., *CA9* was identified as a promising prognostic biomarker for CCA [23]. In the present study, we were engaged in finding novel and effective molecule to better improve the prognosis of CCA patients.

RECK gene is discovered by Takahashi et al. in v-ki-ras-transfected NIH3T3 cells in 1998, and locates on human chromosome 9p12-p13 [24]. *RECK* is about 87 kb in length, including 21 exons and 20 introns. RECK protein consists of 971 amino acids with two hydrophobic regions in the N-terminal and C-terminal, respectively. *RECK* is a novel metastasis suppressor gene, which plays an important role in maintaining the balance of cell proliferation, differentiation and apoptosis. Moreover, *RECK* might be implicated in regulating cell growth and tumor invasion, migration and angiogenesis [25]. In addition, the prognostic role of *RECK* has been identified in several cancers, such as colorectal cancer [26]. So here we attempted to explore the prognostic role of *RECK* in CCA.

In this retrospective study, the expression levels of *RECK* mRNA in CCA tissues and adjacent normal controls were measured using qRT-PCR method. The results showed significantly reduced expression of *RECK* mRNA in CCA samples compared with noncancerous controls, which was consistent with the findings in previous studies. The Chi-square test suggested that *RECK* down-regulation was affected by invasion depth, lymphatic metastasis, differentiation and TNM stage. With these findings, we hypothesized that *RECK* might be related with the CCA development and prognosis. Therefore, the Kaplan-Meier and Cox regression analyses were carried out. It could be concluded from the survival curve that patients with low *RECK* expression were more likely to die than those with high *RECK* expression. The final Cox regression analysis revealed that *RECK* was an independent biomarker for the prognosis of CCA patients with both univariate and multivariate analyses.

Though we have identified the predictive role of *RECK* in CCA, its mechanisms on CCA development and progression have not been well understood. MMPs are composed of over 20 zinc dependent enzymes, which could interact with ECM in tumor cells to destroy connective tissues and induce tumor invasion and migration [27]. Evidence has demonstrated that *RECK* is an inhibitor of MT1-MMP, MMP-2 and MMP-9 and therefore suppresses the development and progression of tumors [19]. In addition, in the study of Huang et al., *RECK* was reported to be a binding site of the *miR-21*, which was associated with cancer cell processes as a tumor suppressor gene [28]. Moreover, there were several investigations indicating that *miR-21* was related with CCA occurrence and progression [29, 30]. Therefore, we conjectured that *RECK* might function on CCA through interacting with *miR-21*. In a word, the precise mechanism of *RECK* on CCA development is complex and more further studies are needed in future.

Conclusions

In summary, *RECK* expression was significantly decreased in CCA tissues compared to adjacent normal controls. Down-regulation of *RECK* was influenced by invasion depth, differentiation, lymphatic metastasis and TNM stage. *RECK* could be used as a candidate biomarker for CCA prognosis.

List Of Abbreviations

protein with Kazal motifs (*RECK*)

cholangiocarcinoma (CCA)

Quantitative real-time PCR (qRT-PCR)

extrahepatic cholangiocarcinoma (EHCC)

intrahepatic cholangiocarcinoma (IHCC)

extracellular matrix (ECM)

Matrix metalloproteinases (MMPs)

glycosyphosphatidylinositol (GPI)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

N.W., Y.L. design of the work; Y.Z., H.C. the acquisition, analysis, X.W., Z.L. interpretation of data; Y.L., Y.Z. the creation of new software used in the work; X.W., Z.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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References

1. Yang JD, Campion MB, Liu MC, Chaiteerakij R, Giama NH, Ahmed Mohammed H, Zhang X, Hu C, Campion VL, Jen J, et al. Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma. Hepatology. 2016;63(1):148–58.
2. Liu M, Liu BL, Liu B, Guo L, Wang Q, Song YQ, Dong LH. Cutaneous metastasis of cholangiocarcinoma. World journal of gastroenterology: WJG. 2015;21(10):3066–71.
3. Wang W, Zhang J, Zhan X, Lin T, Yang M, Hu J, Han B, Hu S. SOX4 is associated with poor prognosis in cholangiocarcinoma. Biochem Biophys Res Commun. 2014;452(3):614–21.
4. Poruk KE, Pawlik TM, Weiss MJ. Perioperative Management of Hilar Cholangiocarcinoma. Journal of gastrointestinal surgery: official journal of the Society for Surgery of the Alimentary Tract. 2015;19(10):1889–99.
5. Yao L, Han C, Song K, Zhang J, Lim K, Wu T. Omega-3 Polyunsaturated Fatty Acids Upregulate 15-PGDH Expression in Cholangiocarcinoma Cells by Inhibiting miR-26a/b Expression. Cancer research. 2015;75(7):1388–98.
6. Kwak TW, Shin HJ, Jeong YI, Han ME, Oh SO, Kim HJ, Kim DH, Kang DH. Anticancer activity of streptochlorin, a novel antineoplastic agent, in cholangiocarcinoma. Drug Des Devel Ther. 2015;9:2201–14.

7. Wu CC, Lin ZY, Kuoc CH, Chuang WL. Clinically acceptable colchicine concentrations have potential for the palliative treatment of human cholangiocarcinoma. Kaohsiung J Med Sci. 2015;31(5):229–34.
8. Mao ZY, Guo XC, Su D, Wang LJ, Zhang TT, Bai L. Prognostic Factors of Cholangiocarcinoma After Surgical Resection: A Retrospective Study of 293 Patients. Medical science monitor: international medical journal of experimental clinical research. 2015;21:2375–81.
9. Kamphues C, Al-Abadi N, Bova R, Rademacher S, Al-Abadi H, Klauschen F, Bahra M, Neuhaus P, Pratschke J, Seehofer D. The DNA index as a prognostic tool in hilar cholangiocarcinoma. Journal of surgical oncology. 2015;112(2):214–8.
10. Saito H, Noji T, Okamura K, Tsuchikawa T, Shichinohe T, Hirano S. A new prognostic scoring system using factors available preoperatively to predict survival after operative resection of perihilar cholangiocarcinoma. Surgery. 2016;159(3):842–51.
11. Xu M, Wang HF, Zhang HZ: **Expression of RECK and MMPs in Hepatoblastoma and Neuroblastoma and Comparative Analysis on the Tumor Metastasis**. *Asian Pacific journal of cancer prevention: APJCP* 2015, **16**(9):4007–4011.
12. Wang L, Wang Q, Li HL, Han LY. Expression of MiR200a, miR93, metastasis-related gene RECK and MMP2/MMP9 in human cervical carcinoma–relationship with prognosis. Asian Pacific journal of cancer prevention: APJCP. 2013;14(3):2113–8.
13. Gomes LR, Fujita A, Mott JD, Soares FA, Labriola L, Sogayar MC. RECK is not an independent prognostic marker for breast cancer. BMC Cancer. 2015;15:660.
14. Zhou DN, Deng YF, Li RH, Yin P, Ye CS. Concurrent alterations of RAGE, RECK, and MMP9 protein expression are relevant to Epstein-Barr virus infection, metastasis, and survival in nasopharyngeal carcinoma. Int J Clin Exp Pathol. 2014;7(6):3245–54.
15. Shi G, Yoshida Y, Yuki K, Nishimura T, Kawata Y, Kawashima M, Iwaisako K, Yoshikawa K, Kurebayashi J, Toi M, et al: **Pattern of RECK CpG methylation as a potential marker for predicting breast cancer prognosis and drug-sensitivity**. *Oncotarget* 2016.
16. Mahl C, Egea V, Megens RT, Pitsch T, Santovito D, Weber C, Ries C. RECK (reversion-inducing cysteine-rich protein with Kazal motifs) regulates migration, differentiation and Wnt/beta-catenin signaling in human mesenchymal stem cells. Cell Mol Life Sci. 2016;73(7):1489–501.
17. Walsh LA, Roy DM, Reyngold M, Giri D, Snyder A, Turcan S, Badwe CR, Lyman J, Bromberg J, King TA, et al. RECK controls breast cancer metastasis by modulating a convergent, STAT3-dependent neoangiogenic switch. Oncogene. 2015;34(17):2189–203.
18. Alexius-Lindgren M, Andersson E, Lindstedt I, Engstrom W. The RECK gene and biological malignancy–its significance in angiogenesis and inhibition of matrix metalloproteinases. Anticancer research. 2014;34(8):3867–73.
19. Zhang Y, Cheng S, Zhang G, Ma W, Liu Y, Zhao R, Zhang Q, Pang D. Low expression of RECK indicates a shorter survival for patients with invasive breast cancer. Cancer Sci. 2012;103(6):1084–9.

20. Wittschieber D, Stenzinger A, Klauschen F, Stephan C, Jung K, Erbersdobler A, Rabien A. Decreased RECK and Increased EMMPRIN expression in urothelial carcinoma of the bladder are associated with tumor aggressiveness. Pathobiol J ImmunoPathol Mol Cell Biol. 2011;78(3):123–31.
21. Rabien A, Ergun B, Erbersdobler A, Jung K, Stephan C. RECK overexpression decreases invasive potential in prostate cancer cells. Prostate. 2012;72(9):948–54.
22. Wu Z, Boonmars T, Nagano I, Boonjaraspinyo S, Srinontong P, Ratasuwan P, Narong K, Nielsen PS, Maekawa Y. Significance of S100P as a biomarker in diagnosis, prognosis and therapy of opisthorchiasis-associated cholangiocarcinoma. International journal of cancer Journal international du cancer. 2016;138(2):396–408.
23. Gu M. CA9 overexpression is an independent favorable prognostic marker in intrahepatic cholangiocarcinoma. Int J Clin Exp Pathol. 2015;8(1):862–6.
24. Takahashi C, Sheng Z, Horan TP, Kitayama H, Maki M, Hitomi K, Kitaura Y, Takai S, Sasahara RM, Horimoto A, et al. Regulation of matrix metalloproteinase-9 and inhibition of tumor invasion by the membrane-anchored glycoprotein RECK. Proc Natl Acad Sci USA. 1998;95(22):13221–6.
25. Trombetta-Lima M, Winnischofer SM, Demasi MA, Astorino Filho R, Carreira AC, Wei B, de Assis-Ribas T, Konig MS, Bowman-Colin C, Oba-Shinjo SM, et al. Isolation and characterization of novel RECK tumor suppressor gene splice variants. Oncotarget. 2015;6(32):33120–33.
26. Stenzinger A, von Winterfeld M, Rabien A, Warth A, Kamphues C, Dietel M, Weichert W, Klauschen F, Wittschieber D. Reversion-inducing cysteine-rich protein with Kazal motif (RECK) expression: an independent prognostic marker of survival in colorectal cancer. Human pathology. 2012;43(8):1314–21.
27. Siddesha JM, Valente AJ, Sakamuri SS, Gardner JD, Delafontaine P, Noda M, Chandrasekar B. Acetylsalicylic acid inhibits IL-18-induced cardiac fibroblast migration through the induction of RECK. Journal of cellular physiology. 2014;229(7):845–55.
28. Huang Q, Liu L, Liu CH, You H, Shao F, Xie F, Lin XS, Hu SY, Zhang CH. MicroRNA-21 regulates the invasion and metastasis in cholangiocarcinoma and may be a potential biomarker for cancer prognosis. Asian Pacific journal of cancer prevention: APJCP. 2013;14(2):829–34.
29. Liu Z, Jin ZY, Liu CH, Xie F, Lin XS, Huang Q. MicroRNA-21 regulates biological behavior by inducing EMT in human cholangiocarcinoma. Int J Clin Exp Pathol. 2015;8(5):4684–94.
30. Chusorn P, Namwat N, Loilome W, Techasen A, Pairojkul C, Khuntikeo N, Dechakhamphu A, Talabnin C, Chan-On W, Ong CK, et al. Overexpression of microRNA-21 regulating PDCD4 during tumorigenesis of liver fluke-associated cholangiocarcinoma contributes to tumor growth and metastasis. Tumour biology: the journal of the International Society for Oncodevelopmental Biology Medicine. 2013;34(3):1579–88.

Tables

Table 1. Relationship between *RECK* expression and clinical characteristics of CCA patients

Clinical features	Case NO.	Expression		$_c2$	*P* value
		Low	High		
Age (years)				1.770	0.183
≦40	63	28	35		
>40	64	36	28		
Sex				2.268	0.132
Male	67	38	29		
Female	60	26	34		
Tumor size (cm)				1.352	0.245
≦3	68	31	37		
>3	59	33	26		
Family history				1.454	0.228
Yes	49	28	21		
No	78	36	42		
Invasion depth				4.945	0.026
Total infiltration	61	37	24		
Local infiltration	66	27	39		
Differentiation				5.014	0.025
Well, moderate	70	29	41		
Poor	57	35	22		
Lymphatic metastasis				6.615	0.010
Positive	65	40	25		
Negative	62	24	38		
TNM stage				5.937	0.015
I,II	73	30	43		
III,IV	54	34	20		

Table 2. Univariate and multivariate analysis of clinical parameters in CCA patients

Clinical features	Univariate		Multivariate	
	P value	HR (95%CI)	*P* value	HR (95%CI)
Invasion depth	0.021	1.712 (1.084-2.704)	-	-
Differentiation	0.042	1.603 (1.018-2.523)	-	-
TNM stage	0.004	1.960 (1.243-3.089)	0.004	1.972 (1.242-3.131)
RECK expression	0.000	5.290 (3.195-8.758)	0.000	5.376 (3.231-8.946)

Figures

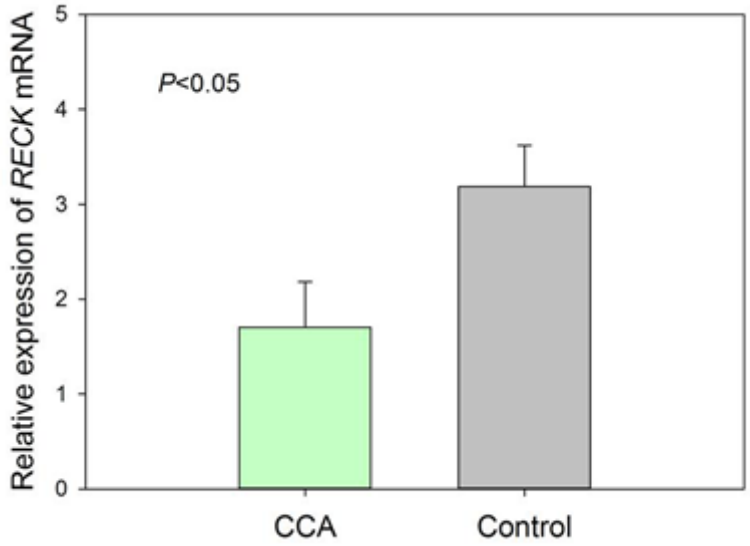

Figure 1

The expression of RECK mRNA was measured by qRT-PCR and normalized to GAPDH. The result showed that RECK mRNA was negatively expressed in CCA tissues compared with adjacent normal controls (P<0.05).

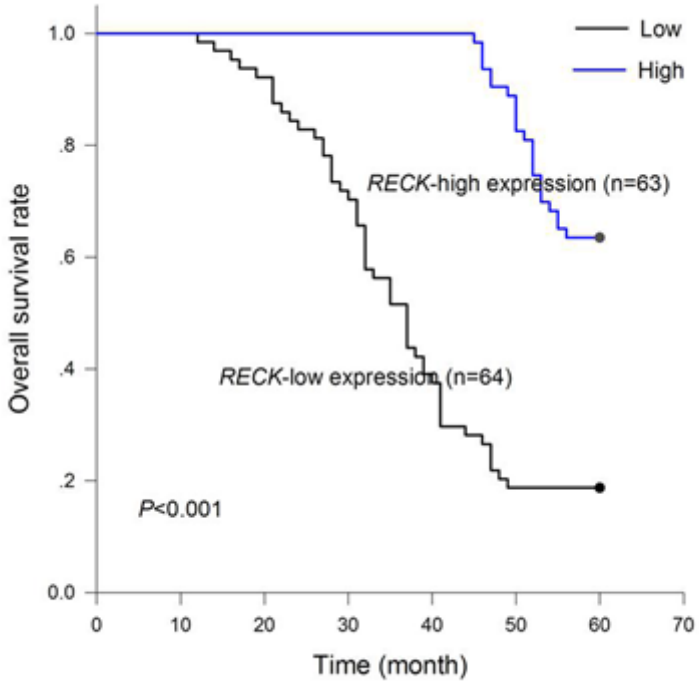

Figure 2

Kaplan-Meier survival curves to describe the overall outcome of CCA patients. The results showed that patients with high RECK expression had a relatively higher overall survival rate than those with low RECK expression levels (P<0.001).