Biochemical and Biophysical Research Communications 464 (2015) 534–540

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Elevated Rictor expression is associated with tumor progression and poor prognosis in patients with gastric cancer

Yuhai Bian [a], Zhengshi Wang [a], Jia Xu [a], Wenyi Zhao [a], Hui Cao [a, *], Zhigang Zhang [b, **]

[a] Department of Gastrointestinal Surgery, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China
[b] State Key Laboratory for Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

ARTICLE INFO

Article history:
Received 24 June 2015
Accepted 1 July 2015
Available online 6 July 2015

Keywords:
Gastric cancer
Rictor
pAktSer473
pS6
Prognosis

ABSTRACT

The rapamycin insensitive companion of mTOR (Rictor) is an essential subunit of mTOR complex 2 (mTORC2), maintains the integrity of the complex and functions as regulator of Akt full activation. Rictor has been implicated to be involved in growth and progression of malignancies, however, little is known about its expression and prognostic role in gastric cancer in particular. Therefore, we investigated the relationship of Rictor expression with clinical outcomes, together with pAktSer473 and pS6, two downstream substrates of mTORC2 and mTORC1, in 396 gastric cancer tissue samples via immunohistochemistry. The results showed that 74.0% and 55.8% of tumors were Rictor and pAktSer473 positive staining, respectively, which correlated well with each other. Patients with positive expressions had poorer overall survival and relapse-free survival compared with those negative staining. Both Rictor and pAktSer473 expression were associated with lymph node metastasis, TNM stage, and WHO grading. Rictor was also correlated with tumor size, depth of invasion, and tumor thrombus, while pAktSer473 was also correlated with distant metastasis. In spite of 67.4% expression rate was presented in gastric cancer tissues, no significant association was observed between pS6Ser235/236, representing mTORC1 activity, and clinicopathological features or prognosis. These results suggest that mTORC2/Rictor/pAkt may play a more important role than mTORC1/pS6 in tumor progression, which could act as a prognostic biomarker or potential therapeutic target in gastric cancer.

1. Introduction

Gastric carcinoma is the fourth most common cancer worldwide and is the third leading cause of cancer-related deaths. It has been estimated that there were more than 723,100 deaths from gastric cancer during 2012, with more than 951,600 new cases diagnosed [1]. Although a steady decline of incidence and mortality rates was observed in western countries, it still remains the second common malignancy in China [2]. The most important factor for survival in gastric cancer patients is tumor stage and the potential for complete resection. However, for patients with advanced or inoperable stage at the time of diagnosis, conventional adjuvant treatment including chemotherapy and radiotherapy is far from satisfactory, resulting in an overall 5-year survival rate of 30%. Therefore, a better understanding of molecular mechanism involved in gastric cancer progression may contribute to identify novel specific diagnostic markers and effective therapeutic targets.

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase which plays a key role in regulation of cellular metabolism, growth and proliferation [3]. Based on sensitivity to rapamycin treatment, mTOR nucleates two structurally and functionally distinct multi-protein complexes known as mTOR complex 1 (mTORC1) and 2 (mTORC2). Raptor (regulatory-associated protein of mTOR) and Rictor (rapamycin insensitive companion of mTOR) are the core proteins for mTORC1 and mTORC2, respectively. Dysregulation of the mTOR signaling pathway has been observed in multiple human cancers [4,5]. Of note, mTOR was found to be activated in gastric cancer, and p-mTOR level was demonstrated to be a prognostic factor, indicating its potential value for target

* Corresponding author. Department of Gastrointestinal Surgery, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, 1630 Dongfang Road, Shanghai 200127, China.
** Corresponding author. State Key Laboratory for Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Room 428, Wenxuan Building of Medicine, 800 Dongchuan Road, Shanghai 200240, China.
E-mail addresses: oceanby@sina.com (Y. Bian), watrudoing@163.com (Z. Wang), xujia201800@126.com (J. Xu), zhaowy2win@163.com (W. Zhao), caohuishcn@hotmail.com (H. Cao), zzhang@shsci.org (Z. Zhang).

http://dx.doi.org/10.1016/j.bbrc.2015.07.001
0006-291X/

therapy [6]. However, despite a seemingly clear rationale for use of mTOR inhibition in gastric cancer, rapamycin analogue everolimus showed less successful benefit in clinical trials than expected as in other tumors [7]. Main reason for this may attribute to different sensitivity of two distinct mTOR complexes. The mTORC1 complex is sensitive to rapamycin treatment and responds to a spectrum of stimuli, mediating phosphorylation and activation of the p70S6 ribosomal kinase (S6K) and the eukaryotic translation initiation factor 4E binding protein-1 (4E-BP1). Major function of mTORC1 is to control protein synthesis and promote anabolic processes [8]. The mTORC2 complex, which is insensitive to rapamycin, plays a role in cell growth and survival by phosphorylation and activation of Akt, regulating cell cycle-dependent actin cytoskeleton changes and cell migration [9]. Therefore, treatment of rapamycin or its analogues dominantly inhibit mTORC1/S6K pathway, and relieves a negative feedback loop from S6K to insulin-like growth factor-1 receptor (IGF-1R), which could transduce signals through intact mTORC2 leading to activating Akt [10]. This paradoxical Akt activation presents a problem as it promotes cell survival and resistance to the therapeutic benefits of mTORC1 inhibition [11].

Rictor is a key component of mTORC2 and is required for its function. mTORC2 has been implicated as the major hydrophobic kinase to phosphorylate Akt at the residue of Ser473, thus placing mTOR both upstream and downstream of Akt [4]. Because Akt activation is widespread in gastric cancer, mTORC2/Rictor might be a promising therapeutic target. Previous research has shown that targeted inhibition of mTORC2 activity through Rictor knockdown induces apoptosis of breast cancer cells and prevents cell migration and metastasis [12,13]. Moreover, ablation of mTORC2 activity by deleting Rictor protects PTEN heterozygous mice from prostate cancer [14]. Targeting Rictor also inhibits colorectal cancer formation and metastasis in vivo [15,16]. Although Rictor has been implicated in progression of a variety of malignancies, its prognostic role and correlation with pAktSer473 in gastric cancer have not been elaborated yet. In addition, the expression of pS6, downstream substrate of mTORC1, remains obscure in gastric cancer, either. The purpose of the present study was to investigate the expression of Rictor, pAktSer473 and pS6 in gastric cancer, their co-expression and association with patients' outcomes.

2. Materials and methods

2.1. Patients and clinical data

A total of 396 patients with gastric carcinoma who underwent surgical resection at Renji Hospital, Shanghai Jiao Tong University School of Medicine between January 2008 and December 2010 were enrolled for this study. Among them, 362 cases without distant metastasis received gastrectomy together with a standard D2 lymph node dissection. The other 34 metastatic patients with primary tumor complications such as obstruction or bleeding underwent palliative stomach resection. Pathological tumor staging referred to the 7th edition of Union for International Cancer Control (UICC) TNM staging system. All participants had complete follow-up. The overall survival (OS) time was determined from the date of surgery to the follow-up deadline or date of death. Relapse-free survival (RFS) time was defined as from the date of surgery to the last observation or the date of clinical recurrence diagnosed by imaging or surgical exploration. The follow-up deadline was August 2014, and the median follow-up period was 49 months (range 2–80 months). The project was approved by Ethics Committee of Renji Hospital, Shanghai Jiao Tong University School of Medicine for the use of samples, and informed consents were obtained from all patients before study.

2.2. Immunohistochemistry

Tissue microarrays were constructed by Suzhou Xinxin Biotechnology Co., Ltd. (Suzhou, China). Prepared slides were incubated at 60 °C for 1 h, deparaffinized with xylene, and rehydrated in a series of graded alcohols. The antigens were retrieved in 0.01M sodium citrate buffer (pH6.0) using a microwave oven, and 3% hydrogen peroxide was used to block endogenous peroxidase activity. After 60 min of preincubation with 10% goat serum to prevent nonspecific binding, the tissue slides were incubated with primary antibody against Rictor (Bethyl Laboratories, #A300-459A, U.S., dilution of 1:400), pAktSer473 (Epitomics, #2118–1, U.S., dilution of 1:100) or pS6Ser235/236 (CST, #2211, U.S., dilution of 1:100) overnight at 4 °C. Next day, the tissues were then incubated with goat anti-rabbit IgG secondary antibody (Abcam, U.K.) for 60 min at room temperature. Immunostaining was carried out with DAB substrate kit (Thermo Scientific, U.S.), followed by immersing into hematoxylin for nuclear counterstaining.

2.3. Scoring of staining

The results of immunohistochemical staining were evaluated by two independent investigators according to a semiquantitative grading system based on both proportion of stained cells and their intensity [15]. The extent of staining was scored as follows: no staining = 0; <1/3 staining = 1; 1/3 to 2/3 staining = 2; and >2/3 staining = 3. Staining intensity was scored as: none = 0; weak = 1; medium = 2; and strong = 3. The intensity and percentage scores were added to give a final score ranging from 0 to 6. The results of immunostaining were divided into two groups, 0–2 was considered negative (−) and 3–6 was considered positive (+).

2.4. Statistical analysis

All statistical analyses were carried out using SPSS software (version 17.0). The associations of Rictor, pAktSer473 or pS6Ser235/236 expression with various clinicopathological parameters were calculated using the chi-square test. Cumulative survival and relapse-free survival were estimated by the Kaplan–Meier method and the log-rank test was used to compare the survival distributions. Univariate analyses were based on a Cox proportional hazard regression model. Multivariate survival analyses were conducted using a Cox regression analysis with a forward stepwise method. All variables statistically significant on univariate analysis were incorporated into the model. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Expression of Rictor and pAktSer473 are correlated with clinicopathological features in patients with gastric cancer

In gastric cancer tissues, Rictor, pAktSer473 and pS6Ser235/236 exhibited cytoplasmic staining, granularly or diffuse distribution, and representative stains were shown in Fig. 1. Positive expression rates of the three proteins were 74.0%, 55.8% and 67.4%, respectively. Clinicopathological characteristics of 396 patients could be referred from Table 1. Statistical analyses indicated that Rictor expression was correlated with age, tumor size, depth of invasion, lymph node metastasis, TNM stage, WHO grading and tumor thrombus. There was no significant association with gender, tumor location, distant metastasis and perineural invasion. Meanwhile, the expression of pAktSer473 was significantly correlated with lymph node or distant metastasis, TNM stage and WHO grading. However, no significant association was observed between

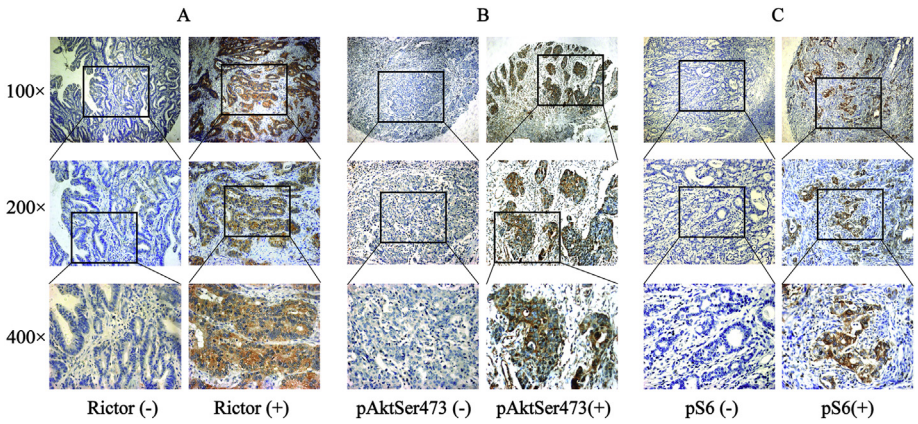

Fig. 1. Representative immunohistochemical stains for Rictor **(A)**, pAktSer473 **(B)**, and pS6Ser235/236 **(C)** in gastric cancer tissues.

pS6Ser235/236 expression and clinicopathological features except for WHO grading. These results suggest that Rictor and pAktSer473 may be implicated in the progression of gastric cancer.

3.2. Rictor positive expression can predict poor prognosis of gastric cancer

Kaplan–Meier survival analysis showed patients with Rictor positive expression were associated with decreased OS and RFS (Figs. 2A and 3A, Table 2). For all 396 patients, the 5-year cumulative survival rate of Rictor negative and positive group was 71.9% and 56.8%, respectively (P = 0.008). Among 362 patients who underwent radical resection, the 5-year relapse-free survival rate was 75.1% and 60.0%, respectively (P = 0.006). However, no statistical significance was observed between Rictor expression and OS or RFS by multivariate analysis (Table 3).

Table 1
Correlation of Rictor, pAktSer473 and pS6Ser235/236 expression with clinicopathological characteristics in 396 gastric cancer patients.

Factors	Cases	Rictor (+)		pAktSer473 (+)		pS6Ser235/236 (+)	
		n (%)	*P* value	n (%)	*P* value	n (%)	*P* value
Gender							
Male	261	196 (75.1)	0.485	154 (59.0)	0.075	176 (67.4)	0.996
Female	135	97 (71.9)		67 (49.6)		91 (67.4)	
Age (yr)							
≤60	177	121 (68.4)	**0.022**	98 (55.4)	0.874	113 (63.8)	0.171
>60	219	172 (78.5)		123 (56.2)		154 (70.3)	
Location							
Upper	25	19 (76.0)	0.968	11 (44.0)	0.216	19 (76.0)	0.283
Central	158	116 (73.4)		96 (60.8)		107 (67.7)	
Lower	207	154 (74.4)		112 (54.1)		139(67.1)	
Diffuse	6	4 (66.7)		2 (33.3)		2 (33.3)	
Size							
<5 cm	204	140 (68.6)	**0.012**	107 (52.2)	0.166	134 (65.7)	0.447
≥5 cm	192	153 (79.7)		114 (59.4)		133 (69.3)	
Depth of invasion							
T1+T2	132	88 (66.7)	**0.019**	69 (52.3)	0.316	94 (71.2)	0.255
T3+T4	264	205 (77.7)		152 (57.6)		173 (65.5)	
Lymph node							
Negative	164	106 (64.6)	**<0.001**	73 (44.5)	**<0.001**	111 (67.7)	0.926
Positive	232	187 (80.6)		148 (63.8)		156 (67.2)	
Distant metastasis							
M0	362	266 (73.5)	0.451	196 (54.1)	**0.030**	244 (67.4)	0.977
M1	34	27 (79.4)		25 (73.5)		23 (67.6)	
TNM stage							
I	100	63 (63.0)	**0.007**	47 (47.0)	**0.004**	69 (69.0)	0.975
II	99	70 (70.7)		47 (47.5)		67 (67.7)	
III	163	133 (81.6)		102 (62.6)		108 (66.3)	
IV	34	27 (79.4)		25 (73.5)		23 (67.6)	
WHO grading							
Grade 1	72	51 (70.8)	**0.017**	34 (47.2)	**0.045**	50 (69.4)	**0.047**
Grade 2	121	101 (83.5)		78 (64.5)		91 (75.2)	
Grade 3	203	141 (69.5)		109 (53.7)		126 (62.1)	
Tumor thrombus							
Negative	342	247 (72.2)	**0.044**	187 (54.7)	0.255	228 (66.7)	0.418
Positive	54	46 (85.2)		34 (63.0)		39 (72.2)	
Perineural invasion							
Negative	370	271 (73.2)	0.201	207 (55.9)	0.835	246 (66.5)	0.133
Positive	26	22 (84.6)		14 (53.8)		21 (80.8)	

Bold means statistically significance, a p value <0.05.

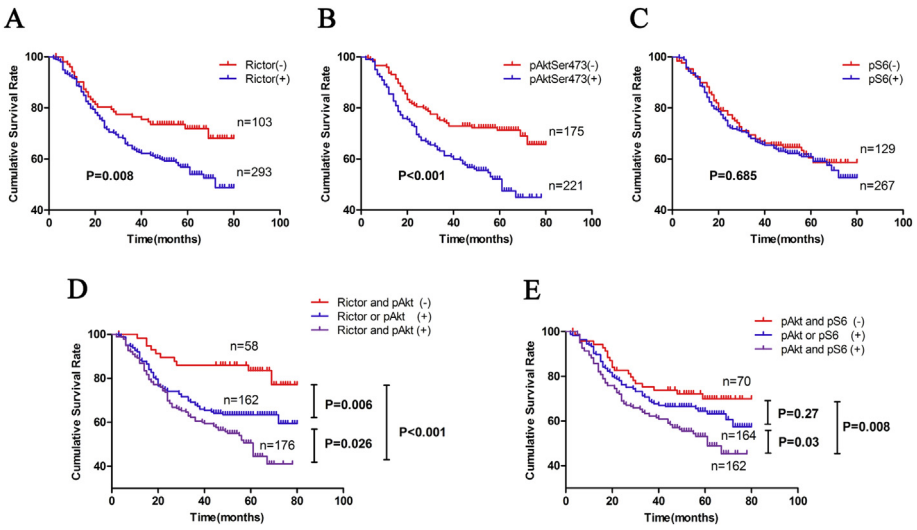

Fig. 2. Kaplan–Meier survival analysis with log-rank test for OS in all 396 patients with gastric cancer sorted by immunostaining of different biomarkers. Rictor or pAktSer473 positive expression predicts poor prognosis **(A, B)**, while no difference was observed between pS6Ser235/236 positive and negative groups **(C)**. Survival durations were significantly worse in patients with either or both positive expression of Rictor and pAktSer473 **(D)**. Combined expression of pAktSer473 and pS6Ser235/236 showed no further prognostic role than pAktSer473 alone **(E)**.

3.3. pAktSer473 expression is associated with Rictor expression and acts as a prognostic indicator for gastric cancer

As pAktSer473 is the major downstream substrate of mTORC2, it represents the activity of mTORC2/Rictor. Our results indicated that pAktSer473 was significantly associated with Rictor expression (Table 4). Kaplan–Meier survival analysis showed sharply decreased OS in pAktSer473 positive individuals compared with negative cases, and the 5-year cumulative survival rate was 52.1% and 71.3%, respectively (Fig. 2B). For RFS, the results were similar, and the 5-year relapse-free survival rate was 58.7% and 70.3%, respectively (Fig. 3B). In univariate analysis, pAktSer473 positive expression predicted poor prognosis (Table 2), and the multivariate Cox proportional hazard model showed a trend of pAktSer473 as an independent prognostic indicator for OS, although the trend did not reach statistical significance (Table 3). In patients with negative staining for both Rictor and pAktSer473, the OS and RFS were significantly better than those with both or either positive for the two biomarkers (Figs. 2D&3D), indicating that detection of Rictor and pAktSer473 simultaneously may predict prognosis more precisely in gastric cancer patients.

3.4. Expression of pS6Ser235/236 presents no relationship with prognosis in gastric cancer

Although quite a part of tumor tissues presented pS6Ser235/236 positive expression, no significant association for prognosis was found between pS6 and gastric cancer by Kaplan–Meier survival analyses (Figs. 2C&3C). The results were repeatedly confirmed by univariate analysis (Table 2). In addition, combined expression of pS6 and pAktSer473, two downstream targets of mTORC1 and

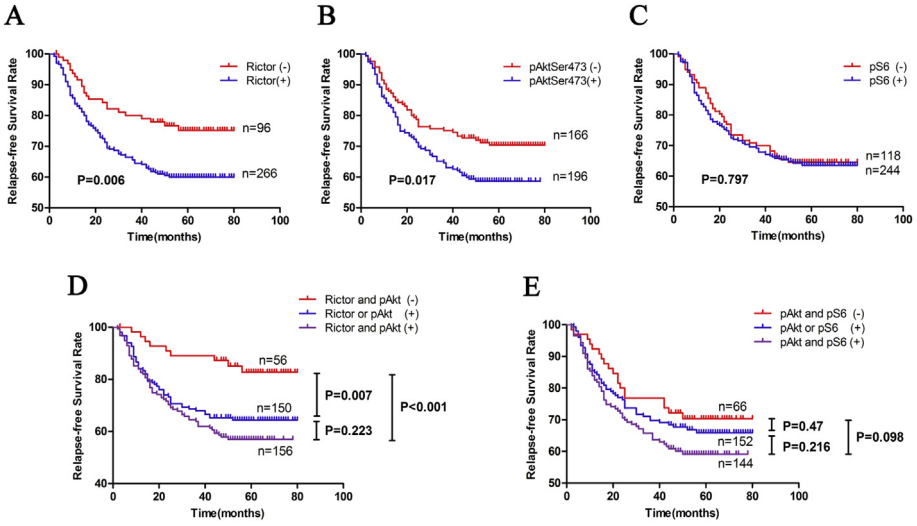

Fig. 3. Kaplan–Meier survival analysis with log-rank test for RFS in 362 patients underwent radical resection sorted by immunostaining of different biomarkers. Rictor or pAktSer473 positive expression predicts shorter RFS **(A, B)**, while no difference was observed between pS6Ser235/236 positive and negative groups **(C)**. Survival durations were significantly worse in patients with either or both positive expression of Rictor and pAktSer473 **(D)**. Combined expression of pAktSer473 and pS6Ser235/236 showed no significant difference in RFS **(E)**.

Table 2
Univariate analysis of overall survival and relapse-free survival in gastric cancer patients according to clinicopathological factors and Rictor/pAktSer473/pS6 expression.

Variable	Overall survival (n = 396)		Relapse-free survival (n = 362)	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Gender	0.891 (0.637–1.247)	0.502	0.793 (0.543–1.159)	0.232
Age	1.154 (0.840–1.585)	0.378	1.047 (0.739–1.483)	0.798
Tumor location	1.225 (0.941–1.596)	0.132	1.167 (0.874–1.558)	0.296
Tumor size	3.620 (2.562–5.116)	**<0.001**	3.352 (2.315–4.853)	**<0.001**
Depth of invasion	8.579 (4.755–15.476)	**<0.001**	8.277(4.457–15.369)	**<0.001**
Lymph node metastasis	7.708 (4.761–12.479)	**<0.001**	7.111 (4.314–11.720)	**<0.001**
Distant metastasis	10.045(6.661–15.149)	**<0.001**		
TNM stage	4.716 (3.683–6.039)	**<0.001**	4.747 (3.346–6.735)	**<0.001**
WHO grading	2.367 (1.821–3.077)	**<0.001**	2.489 (1.859–3.332)	**<0.001**
Tumor thrombus	3.258 (2.254–4.710)	**<0.001**	3.349 (2.220–5.051)	**<0.001**
Perineural invasion	1.923 (1.128–3.278)	**0.016**	2.912 (1.744–4.862)	**<0.001**
Rictor expression	1.713 (1.143–2.567)	**0.009**	1.848 (1.177–2.902)	**0.008**
pAktSer473 expression	1.931 (1.379–2.703)	**<0.001**	1.537 (1.074–2.199)	**0.019**
pS6 expression	1.072 (0.765–1.503)	0.687	1.050 (0.724–1.522)	0.798

Bold means statistically significance, a p value <0.05.

Table 3
Multivariate Cox proportional hazards model to predict factors associated with overall survival and relapse-free survival in gastric cancer patients.

Variable	Overall survival (n = 396)		Relapse-free survival (n = 362)	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Tumor size	1.195 (0.812–1.759)	0.366	1.254 (0.834–1.883)	0.277
Depth of invasion	3.229 (1.703–6.120)	**<0.001**	3.415 (1.756–6.639)	**<0.001**
Lymph node metastasis	3.507 (2.073–5.934)	**<0.001**	3.779 (2.211–6.459)	**<0.001**
Distant metastasis	4.415 (2.870–6.793)	**<0.001**		
WHO grading	1.818 (1.349–2.450)	**<0.001**	2.047 (1.471–2.849)	**<0.001**
Tumor thrombus	1.794 (1.215–2.650)	**0.003**	1.901 (1.232–2.933)	**0.004**
Perineural invasion	1.113 (0.638–1.940)	0.707	1.223 (0.722–2.073)	0.454
Rictor expression	1.203 (0.788–1.836)	0.392	1.311 (0.824–2.088)	0.253
pAktSer473 expression	1.332 (0.943–1.882)	0.104	1.221 (0.847–1.762)	0.285

Bold means statistically significance, a p value <0.05.

Table 4
Association of Rictor expression with Akt activation in 396 gastric cancer patients.

	Number of patients (%)		Total
	pAktSer473(−)	pAktSer473 (+)	
Rictor (−)	58 (56.3)	45 (43.7)	103
Rictor (+)	117 (39.9)	176 (60.1)	293
Total	175	221	***P* = *0.004***

Bold means statistically significance, a p value <0.05.

mTORC2, showed no further prognostic value than pAktSer473 expression alone, either (Figs. 2E&3E).

3.5. Prognostic factors in patients with gastric cancer

Univariate analysis revealed that overall and relapse-free survival time decreased according to larger tumor, deeper invasion, presence of lymph node or distant metastasis, advanced stage of disease, poor differentiation, presence of tumor thrombus or perineural invasion, and positive expression of Rictor or pAktSer473 (Table 2). Furthermore, the multivariate analysis showed that depth of tumor invasion, lymph node or distant metastasis, WHO grading, and tumor thrombus were independent factors for patients with gastric cancer (Table 3).

4. Discussion

mTOR signaling is one of the key pathways that regulates tumorigenesis and progression of malignancies [4,5]. Specific mTOR inhibitors, such as rapamycin analogues, have already achieved clinical efficacy in some cancer types [17,18]. However, the results in gastric cancer have been less successful than expected [7]. Possible explanation for this may include two aspects. First, rapamycin is a universal inhibitor of mTORC1-dependent S6K phosphorylation, but the existence of negative feedback loop from S6K to Akt through IGF-1R presents a paradoxical feedback activation of Akt, which relates to cell survival and chemoresistance. Second, in two mTOR complexes, mTORC2, composed by mTOR and Rictor, is insensitive to rapamycin analogues, and is critical to full activation of Akt. As a major component of mTORC2, Rictor plays a crucial role in cancer proliferation, invasion and metastasis. The present study uncovered a previously unappreciated correlation of Rictor expression and activity with tumor prognosis of gastric cancer patients.

It has been reported that overexpression of Rictor is positively associated with tumor progression and poor survival in colorectal cancer [16], hepatocellular carcinoma [19], endometrial carcinoma [20], and pituitary adenoma [21]. However, the role of Rictor in breast cancer remains controversial [13,22]. Consistent with the majority of these findings, the present work manifested Rictor positivity was directly correlated with tumor size, invasion of stomach wall, infiltration of lymph node and vessels, tumor stage and differentiation, and was inversely correlated with survival rate in Chinese gastric cancer patients. These results suggest that Rictor is implicated in the progression of gastric cancer and might be used as a novel biomarker for prognosis.

Rictor mostly exerts its role as a key scaffold protein binding with mTOR, mLST, mSIN1 and Protor-1 to form mTORC2, which has been implicated as the major kinase to phosphorylate the Ser473 residue of Akt to its full activation. pAktSer473 represents the activity of mTORC2 and Rictor. In colorectal cancer, expression

of Rictor was found to correlate with elevated pAktSer473 expression, and samples stained positive for pAktSer473 exhibited elevated Rictor expression [15]. Here, we also examined the expression of pAktSer473 in the same sets of gastric cancer tissues. Our data are in agreement with previous studies in gastric cancer, reporting that immunostaining of pAktSer473 was observed in 58%–68% of patients, and was significantly related to clinicopathological variables and outcomes [23,24]. In addition, detailed investigation revealed that patients with both Rictor and pAktSer473 positive staining had worse prognosis than patients with both or either negative groups, especially by overall survival analysis containing stage IV patients. Therefore, Rictor/pAktSer473 combined analysis may be a more useful prognostic indicator than either biomarker alone, suggesting the importance of Rictor-mTORC2-Akt signaling pathway in gastric cancer. Recent studies show that Rictor also interacts with a variety of different molecules to exert mTORC2-independent functions in regulating cell migration and epithelial–mesenchymal transition [25,26], which explains the tumors with Rictor positive and pAktSer473 negative cases still progressed in advanced stage.

Another mTOR complex, mTORC1, consists of mTOR, Raptor, mLST8 and two negative regulators, is activated by the PI3K-Akt pathway. Activated mTORC1 phosphorylates downstream effector p70S6K1, which subsequently phosphorylates 40s ribosomal protein S6 (pS6), promoting protein translation and cell growth. pS6 is perhaps the best-known S6K1 substrate and is considered as a biomarker of mTORC1 activity and prediction of mTOR inhibitor treatment efficacy [4]. Recent reports indicated pS6 was involved in cervical carcinogenesis [27], glioma proliferation [28], hepatocellular carcinoma metastasis [29], and predicted poor prognosis of lung adenocarcinoma [30]. Studies in renal cell carcinoma showed stronger pS6 expression was more frequently found in metastatic cases, resulting in successful clinical application of everolimus in advanced renal cell carcinoma [17]. However, despite high cytoplasmic expression of pS6Ser235/236 was shown in 67.4% of tumor samples assessed in this study, no association was observed between its positive staining and clinicopathological features or survival rate. Interestingly, Betts and colleagues reported that the pS6Ser240/244 expression was observed in 73% of gastric or gastroesophageal junction adenocarcinoma, with no association with survival, either [31]. These suggest that mTORC1-pS6 pathway may be active in gastric cancer; however, unlike other malignancies, its prognostic value remains to be elucidated. Although rapamycin analogues can specifically block mTORC1 activity, its clinical efficacy may be attenuated in gastric cancer due to insensitivity of downstream target pS6. Meanwhile, inhibition of mTORC1 leads to Akt activation via a negative feedback loop, and might shift the balance to increase mTORC2 activity [11]. In this study, it is demonstrated that Rictor and downstream pAktSer473 are associated with gastric cancer progression and survival, which provide a rationale for simultaneous inhibition of mTORC2 when using rapamycin analogues. Since mTORC2 can not be completely inhibited by traditional mTOR inhibitors, selective targeting Rictor may present a novel therapeutic strategy for treatment of advanced gastric cancer.

In summary, detection of Rictor and its downstream pAktSer473 expression help to precisely subdivide gastric cancers for different prognosis and probably predict outcomes. Rictor potentially offers clinical value in directing personal treatment for gastric cancer patients in the era of molecular target therapy.

Conflict of interest

None declared.

Acknowledgments

This study was supported by National Natural Science Foundation of China (No. 81272743).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.001.

References

[1] L.A. Torre, F. Bray, R.L. Siegel, et al., Global cancer statistics, 2012, CA, Cancer. J. Clin. 65 (2015) 87–108.
[2] L. Yang, Incidence and mortality of gastric cancer in China, World. J. Gastroenterol. 12 (2006) 17–20.
[3] C. Betz, M.N. Hall, Where is mTOR and what is it doing there? J. Cell. Biol. 203 (2013) 563–574.
[4] D.A. Guertin, D.M. Sabatini, Defining the role of mTOR in cancer, Cancer. Cell. 12 (2007) 9–22.
[5] H. Pópulo, J.M. Lopes, P. Soares, The mTOR signalling pathway in human cancer, Int. J. Mol. Sci. 13 (2012) 1886–1918.
[6] G. Yu, J. Wang, Y. Chen, et al., Overexpression of phosphorylated mammalian target of rapamycin predicts lymph node metastasis and prognosis of chinese patients with gastric cancer, Clin. Cancer. Res. 15 (2009) 1821–1829.
[7] A. Ohtsu, J.A. Ajani, Y.X. Bai, et al., Everolimus for previously treated advanced gastric cancer: results of the randomized, double-blind, phase III GRANITE-1 study, J. Clin. Oncol. 31 (2013) 3935–3943.
[8] M. Laplante, D.M. Sabatini, mTOR signaling in growth control and disease, Cell. 149 (2012) 274–293.
[9] D.D. Sarbassov, D.A. Guertin, S.M. Ali, et al., Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, Science 307 (2005) 1098–1101.
[10] X. Wan, B. Harkavy, N. Shen, et al., Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism, Oncogene 26 (2007) 1932–1940.
[11] J.S. Carew, K.R. Kelly, S.T. Nawrocki, Mechanisms of mTOR inhibitor resistance in cancer therapy, Target. Oncol. 6 (2011) 17–27.
[12] H. Li, J. Lin, X. Wang, et al., Targeting of mTORC2 prevents cell migration and promotes apoptosis in breast cancer, Breast. Cancer. Res. Treat. 134 (2012) 1057–1066.
[13] F. Zhang, X.F. Zhang, M.H. Li, et al., mTOR complex component Rictor interacts with PKCζ and regulates cancer cell metastasis, Cancer. Res. 70 (2010) 9360–9370.
[14] D.A. Guertin, D.M. Stevens, M. Saitoh, et al., mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice, Cancer. Cell. 15 (2009) 148–159.
[15] P. Gulhati, Q. Cai, J. Li, et al., Targeted inhibition of mammalian target of rapamycin signaling inhibits tumorigenesis of colorectal cancer, Clin. Cancer. Res. 15 (2009) 7207–7216.
[16] P. Gulhati, K.A. Bowen, J. Liu, et al., mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways, Cancer. Res. 71 (2011) 3246–3256.
[17] R.J. Motzer, B. Escudier, S. Oudard, et al., Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial, Lancet 372 (2008) 449–456.
[18] M.E. Pavel, J.D. Hainsworth, E. Baudin, et al., Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study, Lancet 378 (2011) 2005–2012.
[19] M. Kaibori, N. Shikata, T. Sakaguchi, et al., Influence of rictor and raptor expression of mTOR signaling on long-term outcomes of patients with hepatocellular carcinoma, Dig. Dis. Sci. 60 (2015) 919–928.
[20] S.Y. Wen, C.H. Li, Y.L. Zhang, et al., Rictor is an independent prognostic factor for endometrial carcinoma, Int. J. Clin. Exp. Pathol. 7 (2014) 2068–2078.
[21] W. Jia, A.J. Sanders, G. Jia, et al., Expression of the mTOR pathway regulators in human pituitary adenomas indicates the clinical course, Anticancer. Res. 33 (2013) 3123–3131.
[22] U. Wazir, R.F. Newbold, W.G. Jiang, et al., Prognostic and therapeutic implications of mTORC1 and rictor expression in human breast cancer, Oncol. Rep. 29 (2013) 1969–1974.
[23] D. Murakami, S. Tsujitani, T. Osaki, et al., Expression of phosphorylated Akt (pAkt) in gastric carcinoma predicts prognosis and efficacy of chemotherapy, Gastric. Cancer 10 (2007) 45–51.
[24] C. Cinti, C. Vindigni, A. Zamparelli, et al., Activated Akt as an indicator of prognosis in gastric cancer, Virchows. Arch. 453 (2008) 449–455.
[25] N.K. Agarwal, C.H. Chen, H. Cho, et al., Rictor regulates cell migration by suppressing RhoGDI2, Oncogene 32 (2013) 2521–2526.
[26] I. Serrano, P.C. McDonald, F.E. Lock, et al., Role of the integrin-linked kinase (ILK)/Rictor complex in TGFβ-1-induced epithelial-mesenchymal transition (EMT), Oncogene 32 (2013) 50–60.

[27] M. Andrikopoulou, N. Salakos, E. Deligeoroglou, et al., The role of mTOR signaling pathway in premalignant and malignant cervical lesions, Eur. J. Gynaecol. Oncol. 36 (2015) 36–43.
[28] L. Annovazzi, M. Mellai, V. Caldera, et al., mTOR, S6 and AKT expression in relation to proliferation and apoptosis/autophagy in glioma, Anticancer. Res. 29 (2009) 3087–3094.
[29] L. Zhou, Y. Huang, J. Li, et al., The mTOR pathway is associated with the poor prognosis of human hepatocellular carcinoma, Med. Oncol. 27 (2010) 255–261.
[30] J.M. McDonald, C.E. Pelloski, A. Ledoux, et al., Elevated phospho-S6 expression is associated with metastasis in adenocarcinoma of the lung, Clin. Cancer. Res. 14 (2008) 7832–7837.
[31] G. Betts, H. Valentine, S. Pritchard, et al., FGFR2, HER2 and cMet in gastric adenocarcinoma: detection, prognostic significance and assessment of downstream pathway activation, Virchows. Arch. 464 (2014) 145–156.