RESEARCH ARTICLE

DEPTOR Expression Negatively Correlates with mTORC1 Activity and Tumor Progression in Colorectal Cancer

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Abstract

The mammalian target of rapamycin (mTOR) signaling pathway is upregulated in the pathogenesis of many cancers, including colorectal cancer (CRC). DEPTOR is an mTOR inhibitor whose expression is negatively regulated by mTOR. However, the role of DEPTOR in the development of CRC is not known. The aim of this study was to investigate the expression of DEPTOR and mTORC1 activity (P-S6) in a subset of CRC patients and determine their relation to tumor differentiation, invasion, nodal metastasis and disease-free survival. Here, Immunohistochemical expression of P-S6 (S235/236) and DEPTOR were evaluated in 1.5 mm tumor cores from 90 CRC patients and in 90 samples of adjacent normal mucosa by tissue microarray. The expression of P-S6 (S235/236) was upregulated in CRC, with the positive rate of P-S6 (S235/236) in CRC (63.3%) significantly higher than that in control tissues (36.7%, 30%) ($p<0.05$). P-S6 (S235/236) also correlated with high tumor histologic grade ($p=0.002$), and positive nodal metastasis ($p=0.002$). In contrast, the expression level of DEPTOR was correlated with low tumor histological grade ($p=0.006$), and negative nodal metastasis ($p=0.001$). Interestingly, P-S6 (S235/236) expression showed a significant negative association with the expression of DEPTOR in CRC ($p=0.011$, R= -0.279). However, upregulation of P-S6 (S235/236) ($p=0.693$) and downregulation of DEPTOR ($p=0.331$) in CRC were not significantly associated with overall survival. Thus, we conclude that expression of DEPTOR negatively correlates with mTORC1 activity and tumor progression in CRC. DEPTOR is a potential marker for prognostic evaluation and a target for the treatment of CRC.

Keywords: Colorectal cancer - mTOR - DEPTOR - P-S6 - immunohistochemistry

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Introduction

Colorectal cancer (CRC) is the third most common malignancy and represents 4% of all tumors (Ostendorff et al., 2013). Although 70% of CRC cases undergo curative surgery, 50% of surgically cured patients will have an advanced local recurrence or metastases. CRC is a common cancer with a poor prognosis and significant mortality worldwide (Huh et al., 2009). The extent of local invasion and tumor metastasis are important factors in determining disease outcome. Distant metastases increase treatment failure, and patients are then subjected to palliative treatment (Kunimura et al., 2009). To predict disease outcome and improve therapeutic interventions, continuous efforts are needed to identify more prognostic markers.

The mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine/threonine kinase that integrates signals from growth factors, nutrients, and stresses to regulate multiple processes, including mRNA translation, cell cycle progression, autophagy, and cell survival (Sarbassov et al., 2005; Jacobs et al., 2013; Sarkar., 2013). It is increasingly apparent that deregulation of the mTOR pathway occurs in common diseases, including cancer, emphasizing the importance of identifying and understanding the function of the components of the mTOR signaling network (Guertin et al., 2007). The activation of p70 S6K1 and S6 by mTOR enhances the translation of mRNAs that bear a 50 terminal oligopyrimidine tract, which encodes proteins related to the translational apparatus such as ribosomal proteins, elongation factors eEF1A, eEF2 and the poly A-binding protein (Bjornsti et al., 2004; Mueller et al., 2012). The phosphorylation status of p70S6K (T389) and S6 (S235/236) is commonly used as a marker of mTOR activity and for pharmacodynamic monitoring of mTOR inhibition (Mueller et al., 2012; Sun et al., 2013). Moreover, scientists have identified DEPTOR, also called DEPDC6, as an mTOR-interacting protein whose expression is negatively regulated by mTORC1

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and mTORC2. Loss of DEPTOR activates S6K1, Akt, and SGK1; promotes cell growth and survival; and activates mTORC1 and mTORC2 kinase activities. DEPTOR overexpression suppresses S6K1, but by relieving feedback inhibition from mTORC1 to PI3K signaling, activates Akt (Zhao et al., 2011; Wang et al., 2012; Meng et al., 2013). Consistent with many human cancers with activated mTORC1 and mTORC2 pathways, DEPTOR has low expression in most cancers (Peterson et al., 2009). mTOR has been proved to be one of the most important targets in cancer therapy, and its inhibitors have exhibited encouraging effects in animal experiments and clinical trials (Bjornsti et al., 2004; Baldo et al., 2008). However, the expression of DEPTOR and mTORC1 activity in CRC tissue and their relationship to the prognosis of CRC patients is not known.

In this study, we examined the expression of mTOR-related proteins such as P-S6 (S235/236) and DEPTOR in a subset of primary CRC patients using immunoreactive scores and determined their relation to tumor differentiation, invasion, nodal metastasis, clinicopathologic features and prognosis. From these results, we attempted to evaluate the role of DEPTOR and the mTOR pathway in the development of CRC and its prognosis.

Materials and Methods

Samples and tissue microarray

The tissue microarray was obtained from Shanghai Outdo Biotech Company and contained 90 samples of CRC and 90 samples of adjacent normal mucosal tissue. The 90 patients who were diagnosed with CRC underwent surgical procedures such as right hemicolectomy, left hemicolectomy, sigmoidectomy, anterior resection, or abdominoperineal resection and had no prior history of neo-adjuvant chemotherapy. Samples from these patients were included in this study. All cases were reviewed and classified according to the criteria of the National Comprehensive Cancer Network (NCCN) classification. Pathologic staging was reviewed based on the Tumor Node Metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC 7th Edition) (Edge et al., 2010). The patients were grouped according to age, gender, tumor location, TNM stage, tumor invasion, presence of lymph node metastasis, presence of distant metastasis, and histologic grade. The mean follow-up period was 5.3-6.1 years (operation time from 2006.7 to 2007.5, the last follow-up time was 2012.8). The median age of the patients was 70 years (range: 24 to 90 years). Representative portions of tumor and normal mucosa were marked for tissue microarray construction. One 1.5 mm core of tumor per case and one 1.5 mm core of representative normal mucosa were sampled for tissue microarray. The tumor tissue was cut into 4 μm slices for immunohistochemical staining. All information was provided by Shanghai Outdo Biotech Company. Table 1 shows the clinicopathologic characteristics of this subset of patients.

Immunohistochemistry and Evaluation of Staining

A standard streptavidin-biotin peroxidase complex method was used. After deparaffinization and rehydration, slides were heated in a microwave oven for 15 minutes in 10 mM citrate buffer (pH 6.0) and treated with 3% hydrogen peroxide for 20 minutes. The following antibodies were used: P-S6 (S235/S236) (1:50, Cell Signaling Technology) and DEPTOR (1:50, Genetech Company). Each case was evaluated by estimating the percentages and intensity of tumor cells showing a cytoplasm staining pattern. For immunohistochemistry assessment, the entire tissue section was scanned to assign scores. The staining intensity was scored as 0 (achromatic color), 1 (pallide-flavens), 2 (deep yellow), or 3 (brown). The extent of staining was scored as 0 (<5%), 1 (5%-25%), 2 (26%-50%), 3 (51%-75%), or 4 (>75%), according to the percentages of positively stained areas in relation to the whole carcinoma area (or entire section for normal samples). The sum of the staining intensity and extent scores were used as the final staining scores (0-8) for P-S6/DEPTOR. For the purpose of statistical evaluation, tumors with a final staining score of 0 were considered negative (-), 1-2 were weakly positive (+), 3-5 were moderately positive (++) and 6-8 were strongly positive (+++) (Friedrichs et al., 1993; Ye et al., 1995). P-S6 and DEPTOR immunostaining was evaluated independently by two pathologists blinded to the clinical parameters.

Statistical analysis

All statistical analyses were performed using the SPSS 17.0 statistical software. Statistical significance was determined at $p<0.05$, and tests were two sided. Differences between two groups of cases for one variable were tested using the Mann-Whitney test. The Kruskal-Wallis test was used to test the association between three groups of cases for one independent variable. Differences in expression between normal mucosa and tumor were tested using the Wilcoxon signed-rank test. Correlations between expression of P-S6 and DEPTOR were analyzed

Table 1. Clinicopathologic Characteristics of 90 Cases of Colorectal Cancer

Item		Percantage(%)
Gender	Male	47/90(52.2%)
	Female	43/90(47.8%)
Age	≤70 years	47/90(52.2%)
	>70 years	43/90(47.8%)
Tumor	Right colon	40/90(44.4%)
	Left colon	50/90(55.6%)
	Rectum	0/90(0%)
Primary tumor	T1	4/90(4.4%)
	T2	6/90(6.7%)
	T3	68/90(75.6%)
	T4	12/90(13.3%)
Tumor grade	I-II	54/90(60.0%)
	III-IV	36/90(40.0%)
Tumor size	<5cm	38/90(42.2%)
	≥5cm	52/90(57.8%)
Nodal metastasis	Positive	33/90(36.7%)
	Negative	48/90(53.3%)
	Date not applicable	9/90(10.0%)
Status at end point	Died of disease	44/90(48.9%)
	Alive	46/90(51.1%)

by Pearson's χ^2 tests. Overall survival was calculated as the time from the date of surgery to the date of death or final contact. Univariate and multivariate analyses of overall survival were performed using the Cox proportional hazard model.

Results

Expression of P-S6 (S235/236) and DEPTOR, and Correlations with Clinicopathologic Characteristics

The expression of P-S6 (S235/236) and DEPTOR in normal colorectal mucosa and in 90 patients with CRC was observed using the cytoplasm staining pattern, respectively. In CRC, the expression of DEPTOR in both cytoplasm and brush border was less with reduced staining compared with P-S6. The positive expression of P-S6 and DEPTOR in CRC was 57 (63.3%) and 53 (58.9%), respectively. The sampled normal mucosa showed weak expression with cytoplasm staining (Figure 1).

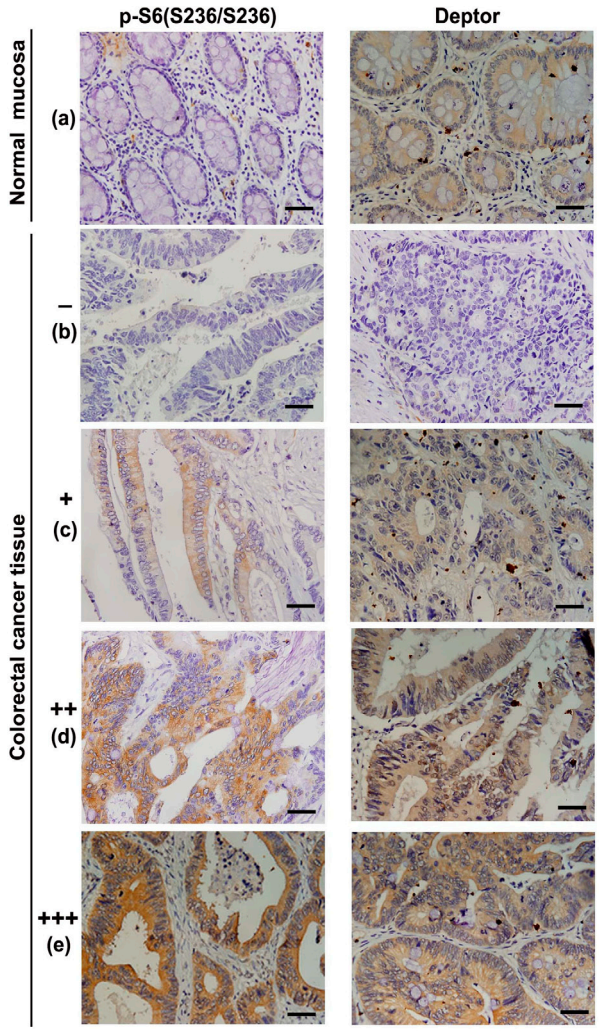

Figure 1. Immunohistochemical Expression of P-S6 (S235/236) and DEPTOR in Normal Colorectal Mmucosa and Colorectal Cancer. Low expression of P-S6 (S235/236) and DEPTOR was found in normal mucosa. In CRC tissue, P-S6 (S235/236) expression showed strong staining in both cytoplasm and brush border. For DEPTOR, staining is shown in both cytoplasm and brush border (mixed pattern) with reduced extent and staining compared with P-S6 (S235/236). (a) Normal colorectal mucosa; (b)-(e) Expression of *p*-S6 and DEPTOR in colorectal cancer tissue: (-) negative; (+) weakly positive; (++) moderately positive; (+++) strongly positive. Scale bar = 50 μm

A high level of P-S6 protein expression was observed in tumor samples (63.3% of cases) compared with adjacent normal mucosa (36.7% of cases) ($p<0.001$). However, the expression of DEPTOR in tumor samples and adjacent normal mucosa was not significantly different ($p=0.093$) (Table 2).

The distribution of positive immunoexpression in CRC showed that DEPTOR and P-S6 were not significantly related to clinicopathologic features such as gender, age, tumor location, tumor size, invasion, depth of primary tumor and status at end point ($p>0.05$). However, the altered expression of P-S6 was correlated with high tumor histologic grade ($p=0.002$) and positive nodal metastasis ($p=0.002$). In contrast, the altered expression of DEPTOR was correlated with low tumor histologic grade ($p=0.006$) and negative nodal metastasis ($p=0.001$) (Table 3).

Correlation between P-S6 and DEPTOR expression.

The expression of P-S6 showed a significant negative

Table 2. Scale of *p*-S6 and DEPTOR Immunoexpression

Scale	*p*-S6			DEPTOR		
	Tumor	Normal mucosa	*p* value	Tumor	Normal mucosa	*p* value
Negative(-)	33/90	69/90	<0.01	37/90	36/90	0.093
Low(+)	28/90	19/90		17/90	39/90	
Moderate(++)	24/90	2/90		34/90	15/90	
High(+++)	5/90	0/90		2/90	0/90	

*Wilcoxon signed-rank test was used to test the difference expression between normal mucosa and tumor

Table 3. Distribution of *p*-S6 and DEPTOR and Positive Immunoexpression in Relation to Clinicopathological Characteristics

	p-S6		DEPTOR	
	Positive (%)	*p* value	Positive (%)	*p* value
Gender				
Male	28/47(59.6)	0.027	25/47(53.2)	0.827
Female	17/43(39.5)		26/43(60.5)	
Age				
≤70years	28/47(59.6)	0.447	25/47(53.2)	0.971
>70years	27/43(62.8)		24/43(55.8)	
Tumor				
Right colon	28/40(70)	0.124	21/40(52.5)	0.478
Left colon	27/50(54)		30/50(60)	
Primary tumor				
T1	2/4(50.0)	0.786*	2/4(50.0)	0.940*
T2	3/6(50.0)		3/6(50.0)	
T3	45/68(66.2)		39/68(57.4)	
T4	6/12(50.0)		7/12(58.3)	
Tumor grade				
I-II	26/54(48.2)	0.002	37/54(68.5)	0.006
III-IV	28/36(77.8)		13/36(36.1)	
Tumor size				
<5cm	22/38(57.9)	0.49	21/38(55.3)	0.706
≥5cm	34/52(65.4)		31/52(59.6)	
Nodal metastasis				
Positive	27/33(59.5)	0.002	12/33(36.4)	0.001
Negative	23/48(47.9)		36/48(75.0)	
Status at end point				
Died of disease	26/44(59.1)	0.702	20/44(45.5)	0.096
Alive	29/46(63.0)		29/46(63.0)	

The distribution is presented as percentage (%), *The Kruskal-Wallis test; other test: The Mann-Whitney test

correlation with the expression of DEPTOR in CRC (p=0.011, R= -0.279) (Table 4).

Survival analysis

A Cox proportional hazard model was used in the univariate analysis of overall survival (Table 5). Of the analyzed clinicopathologic features, older age (≥70 years) (p=0.025), high TNM stage (p<0.001), left-sided tumor (p=0.047), large tumor size (p=0.034), presence of lymph node metastasis (p<0.01), and poorly differentiated histologic grade (stages III and IV) (p<0.01) were significantly associated with shorter overall survival. However, the overex-pression of P-S6 (p=0.693) and low expression of DEPTOR (p=0.331) were not associated with overall survival. In addition, gender (p=0.749) was not associated with overall survival.

The factors considered in multivariate analysis were age, tumor location, tumor size, histologic grade, nodal metastasis and the expression of P-S6 and DEPTOR (Table 6). In this analysis, age, tumor size, histologic grade, and nodal metastasis were independent prognostic factors significantly associated with overall survival.

Table 4. Expression Status and Correlation of *p*-S6 and DEPTOR

DEPTOR	*p*-S6		*p* value
	Positive	Negative	
Positive	26	24	0.011
Negative	27	7	

*Correlation between *p*-S6 and DEPTOR was analyzed by Pearson's $\chi 2$ tests. The *p* value between *p*-S6 and DEPTOR is 0.011, R=-0.279

Table 5. Clincopathologic Characteristics and their Overall Survival by Univariate Cox Proportional Hazards Regression Analysis

Characteristic	NO. of patients	Overall survival	
		HR(95%CI)	*p* value
Gender			
Male	47	1	
Female	43	2.763(1.532-3.994)	0.749
Age			
≤70years	47	1	
>70years	43	1.765(1.521-2.009)	0.025
Tumor location			
Right colon	40	1	
Left colon	50	2.695(0.896-7.545)	0.047
Primary tumor			
T1	4		
T2	6	1	
T3	68	1.213(0.671-3.162)	<0.01
T4	12		
Tumor grade			
I-II	54	1	
III-IV	36	1.543(1.215-1.554)	<0.01
Tumor size			
<5cm	38	1	
≥5cm	52	2.234(1.862-3.347)	0.034
Nodal metastasis			
Positive	33	1	
Negative	48	1.852(0.527-3.752)	<0.01
p-S6			
Positive	57	1	
Negative	33	2.443(0.976-3.655)	0.693
DEPTOR			
Positive	53	1	
Negative	37	1.542(0.261-3.211)	0.331

*HR: hazard ratio; CI: confidence interval

Table 6. Multivariate Analysis for Overall Survival by Cox Proportional Hazards Regression Analysis

Characteristic	NO. of patients	Overall survival	
		HR(95%CI)	*p* value
Age			
≤70years	47	1	
>70years	43	0.867(0.325-1.409)	0.033
Tumor location			
Right colon	40	1	
Left colon	50	2.05(1.121-3.747)	0.422
Tumor grade			
I-II	54	1	
III-IV	36	0.213(0.110-1.230)	<0.001
Tumor size			
<5cm	38	1	
≥5cm	52	0.420(0.220-0.802)	0.009
Nodal metastasis			
Positive	33	1	
Negative	48	0.473(0.263-1.923)	<0.01
p-S6			
Positive	57	1	
Negative	33	2.315(1.651-2.786)	0.752
DEPTOR			
Positive	53	1	
Negative	37	1.277(1.117-1.452)	0.609

Discussion

The occurrence and development of CRC is the outcome of the combined action of many steps, including genetic expression of oncogenes and tumor suppressor genes in cells with an imbalance in apoptosis caused by excessive proliferation, which is one of the most important mechanisms. The importance of the PI3K/Akt/mTOR signaling network in CRC etiology has been noted recently (Martelli et al., 2011). Inhibitors of PI3K/Akt/mTOR signaling have been suggested as potential therapeutic preparations in CRC (Pandurangan., 2013). However, the way in which mTOR signaling pathway-related proteins influence the prognosis of CRC is still unclear. The present study examined the immunohistochemical expression of P-S6 (S235/236) and DEPTOR in human CRC and their relationships with clinicopathologic factors and prognostic significance.

As mentioned above, P-S6 (S235/236) is the downstream signal/target of mTORC1 (Hay et al., 2004). Direct targets of mTORC1 include ribosomal protein S6 kinase 1 (S6K1) which regulate protein translation (Fasolo et al., 2012). In addition, P-S6 (S235/236) is the downstream signal/target of S6K1. S6K1 and S6K2 have been shown to be upregulated in breast cancer (Lyzogubov et al., 2005), and in other cancers. Our study also revealed that expression of P-S6 (S235/236) was significantly higher in CRC tissue than in normal colorectal mucosa (p<0.01). When greatly overexpressed, DEPTOR inhibits mTORC1 (Peterson et al., 2009; Bruneau et al., 2013; Meng et al., 2013; Zhang et al., 2013). In this study, DEPTOR was equally expressed in CRC and adjacent

normal colorectal mucosa. A negative correlation between P-S6 and DEPTOR (p=0.011, R=-0.279) was observed in CRC tissue. Therefore, we can deduce that the PI3K/AKT/mTORC1 pathway is overactive in human CRC.

Previous studies reported that the overexpression of mTOR and P-mTOR may play an important role in colorectal carcinogenesis in relation to the degree of differentiation, invasiveness and metastasis (Wang et al., 2011; Nelson et al., 2012). Our study examined the expression of P-S6 (S235/236), DEPTOR and their relationship to clinicopathologic characteristics. The altered expression of P-S6 (S235/236) was correlated with high tumor histological grade and positive nodal metastasis. In contrast, the altered expression of DEPTOR was correlated with low tumor histological grade and negative nodal metastasis (Table 3). Therefore, these findings indicated that activation of the mTORC1 pathway plays an important role in colorectal carcinogenesis in relation to the degree of differentiation, invasiveness and metastasis.

A recent study revealed that mTOR overexpression was related to poor prognosis in patients with CRC (Iwaya et al., 2012; Mueller et al., 2012; Cai et al., 2014). It was recently demonstrated that mTOR inhibitors have anti-tumor effects (Bijnsdorp et al., 2011; Finn., 2012; Migliardi et al., 2012). However, our study failed to determine the relationship between the expression of P-S6 (S235/236), DEPTOR and overall survival in CRC. Older age (≥70 years), high TNM stage, left-sided tumor, large tumor size, presence of lymph node metastasis, and poorly differentiated histologic grade (stages III and IV) were significantly associated with shorter overall survival.

Recently, many more cancer-related genes or proteins were considered as prognostic markers for CRC, naming activating transcription factor 1 (ATF1) (Huang et al., 2012), Wip1 (Li et al., 2013), CEA and CA 19-9 (Sisik et al., 2013), POK erythroid myeloid ontogenic factor (Pokemon) (Zhao et al., 2013) and nucleophosmin (NPM) (Yang et al., 2014). All these conclusions, of course including ours, were based on the immunohistochemical analysis of the tumor tissues of CRC patients. However, it was uncertain that the aberrant protein expression of the genes above was the cause or the effect for the carcinogenesis of CRC, and the associated cellular and molecular mechanisms were also not clear. Thus, subsequent experiments were designed to further explore the roles of deptor in a mouse CRC model induced by azoxymethane/dextran sodium sulfate (AOM/DSS) in combination with Cre-loxp system (Car1-cre, deptor-loxp).

In summary, our results demonstrate that P-S6 (S235/236) was greatly activated in CRC tissues, and was positively correlated with high tumor histological grade and positive nodal metastasis. In contrast, DEPTOR exhibited the opposite expression and action pattern, which confirmed its inhibitory effect on mTOR signaling and potential tumor suppressor role in CRC. Together, our findings suggest that expression of DEPTOR negatively correlates with mTORC1 activity and tumor progression in CRC. DEPTOR is a potential marker for prognostic evaluation and a target for the treatment of CRC.

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References

Baldo P, Cecco S, Giacomin E, et al (2008). mTOR pathway and mTOR inhibitors as agents for cancer therapy. *Curr Cancer Drug Targets*, **8**, 647-65.

Bijnsdorp IV, Peters GJ (2011). Deoxyribose protects against rapamycin-induced cytotoxicity in colorectal cancer cells *in vitro*. *Nucleosides Nucleotides Nucleic Acids*, **30**, 1197-202.

Bjornsti MA, Houghton PJ (2004). The TOR pathway: a target for cancer therapy. *Nat Rev Cancer*, **4**, 335-48.

Bruneau S, Nakayama H, Woda CB, Flynn EA, Briscoe DM (2013). DEPTOR regulates vascular endothelial cell activation and proinflammatory and angiogenic responses. *Blood*, **122**, 1833-42.

Cai Z, Ke J, He X, et al (2014). Significance of mTOR Signaling and Its Inhibitor Against Cancer Stem-Like Cells in Colorectal Cancer. *Ann Surg Oncol*, **21**, 179-88.

Edge, SB, Compton CC (2010). The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*, **17**, 1471-4.

Fasolo A, Sessa C (2012). Targeting mTOR pathways in human malignancies. *Curr Pharm Des*, **18**, 2766-77.

Finn RS (2012). Current and future treatment strategies for patients with advanced hepatocellular carcinoma: role of mTOR inhibition. *Liver Cancer*, **1**, 247-56.

Friedrichs K, Gluba S, Eidtmann H, Jonat W (1993). Overexpression of p53 and prognosis in breast cancer. *Cancer*, **72**, 3641-7.

Guertin DA, Sabatini DM (2007). Defining the role of mTOR in cancer. *Cancer Cell*, **12**, 9-22.

Hay N, Sonenberg N (2004). Upstream and downstream of mTOR. *Genes Dev*, **18**, 1926-45.

Huang GL, Guo HQ, Yang F, et al (2012).Activating transcription factor 1 is a prognostic marker of colorectal cancer. *Asian Pac J Cancer Prev*, **13**, 1053-7.

Huh JW, Kim HR, Kim YJ, et al (2009). Expression of standard CD44 in human colorectal carcinoma: association with prognosis. *Pathol Int*, **59**, 241-6.

Iwaya T, Yokobori T, Nishida N, et al (2012). Downregulation of miR-144 is associated with colorectal cancer progression via activation of mTOR signaling pathway. *Carcinogenesis*, **33**, 2391-7.

Jacobs BL, Goodman CA, Hornberger TA (2013). The mechanical activation of mTOR signaling: an emerging role for late endosome/lysosomal targeting. *J Muscle Res Cell Motil*, [Epub ahead of print].

Kunimura T, Yoshida T, Suqiyama T, Morohoshi T (2009). The Relationships Between Loss of Standard CD44 Expression and Lymph Node, Liver Metastasis in T3 Colorectal Carcinoma. *J Gastrointest Cancer*, **40**, 115-8.

Li ZT, Zhang L, Gao XZ, Jiang XH, Sun LQ (2013). Expression and significance of the Wip1 proto-oncogene in colorectal cancer. *Asian Pac J Cancer Prev*, **14**, 1975-9.

Lyzogubov V, Khozhaenko Y, Usenko V, et al (2005). Immunohistochemical analysis of Ki-67, PCNA and S6K1/2 expression in human breast cancer. *Exp Oncol*, **27**, 141-4.

Martelli AM, Evangelisti C, Follo MY, et al (2011). Targeting the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in cancer stem cells. *Curr Med Chem*, **18**, 2715-26.

Meng ZX, Li S, Wang L, et al (2013). Baf60c drives glycolytic metabolism in the muscle and improves systemic glucose

homeostasis through Deptor-mediated Akt activation. *Nat Med*, **19**, 640-5.

Migliardi G, Sassi F, Torti D, Trusolino L, Bertotti A (2012). Inhibition of MEK and PI3K/mTOR suppresses tumor growth but does not cause tumor regression in patient-derived xenografts of RAS-mutant colorectal carcinomas. *Clin Cancer Res*, **18**, 2515-25.

Mueller S, Phillips J, Onar-Thomas A, et al (2012). PTEN promoter methylation and activation of the PI3K/Akt/mTOR pathway in pediatric gliomas and influence on clinical outcome. *Neuro Oncol*, **14**, 1146-52.

Nelson LD, Bender C, Mannsperger H, et al (2012). Triplex DNA-binding proteins are associated with clinical outcomes revealed by proteomic measurements in patients with colorectal cancer. *Mol Cancer*.

Ostendorff HP, Awad A, Braunschweiqer KI, et al (2013). Multiplexed veracode bead-based serological immunoassay for colorectal cancer. *J Immunol Methods*, **400-401**, 58-69.

Pandurangan AK (2013). Potential targets for prevention of colorectal cancer: a focus on PI3K/Akt/mTOR and Wnt pathways. *Asian Pac J Cancer Prev*, **14**, 2201-5.

Peterson TR, Laplante M, Thoreen CC, et al (2009). DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell*, **137**, 873-86.

Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*, **307**, 1098-101.

Sarkar S (2013). Regulation of autophagy by mTOR-dependent and mTOR-independent pathways: autophagy dysfunction in neurodegenerative diseases and therapeutic application of autophagy enhancers. *Biochem Soc Trans*, **41**, 1103-30.

Sisik A, Kaya M, Bas G, Basak F, Alimoglu O (2013). CEA and CA 19-9 are still valuable markers for the prognosis of colorectal and gastric cancer patients. *Asian Pac J Cancer Prev*, **14**, 4289-94.

Sun YX, Mao X, Xie L, et al (2013). Differential Activation of mTOR Complex 1 Signaling in Human Brain with Mild to Severe Alzheimer's Disease. *J Alzheimers Dis*, **38**, 437-44.

Wang D, Chen J, Guo F, et al (2011). Clinical significance of mTOR and *p*-mTOR protein expression in human colorectal carcinomas. *Asian Pac J Cancer Prev*, **12**, 2581-4.

Wang Z, Zhong J, Inuzuka H, et al (2012). An evolving role for DEPTOR in tumor development and progression. *Neoplasia*, **14**, 368-75.

Yang YF, Zhang XY, Yang M, et al (2014). Prognostic role of nucleophosmin in colorectal carcinomas. *Asian Pac J Cancer Prev*, **15**, 2021-6.

Ye C, Kiriyama K, Mistuoka C, et al (1995). Expression of E-selectin on endothelial cells of small veins in human colorectal cancer. *Int J Cancer*, **61**, 455-60.

Zhang HR, Chen JM, Zeng ZY, Que WZ (2013). Knockdown of DEPTOR inhibits cell proliferation and increases chemosensitivity to melphalan in human multiple myeloma RPMI-8226 cells via inhibiting PI3K/AKT activity. *J Int Med Res*, **41**, 584-95.

Zhao GT, Yang LJ, Li XX, Cui HL, Guo R (2013). Expression of the proto-oncogene Pokemon in colorectal cancer--inhibitory effects of ansiRNA. *Asian Pac J Cancer Prev*, **14**, 4999-5005.

Zhao Y, Xiong X, Sun Y (2011). DEPTOR, an mTOR inhibitor, is a physiological substrate of SCF(betaTrCP) E3 ubiquitin ligase and regulates survival and autophagy. *Mol Cell*, **44**, 304-16.