EUROPEAN JOURNAL OF CANCER 46 (2010) 3445–3452

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Localisation of phosphorylated mTOR expression is critical to tumour progression and outcomes in patients with endometrial cancer

Yoshio Yoshida *, *Tetsuji Kurokawa, Yuko Horiuchi, Yoko Sawamura, Akiko Shinagawa, Fumikazu Kotsuji*

Department of Gynecology, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuoka-Shimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan

ARTICLE INFO

Article history:
Received 29 June 2010
Received in revised form 25 August 2010
Accepted 2 September 2010

Keywords:
Endometrial endometrioid adenocarcinoma
Phospho-mTOR
Phospho-Akt
Outcome
Location

ABSTRACT

Correlations between mammalian target of rapamycin (mTOR) expression, and clinicopathological features, outcome and Akt expression in endometrial endometrioid adenocarcinoma (EEC) were investigated. Tumour samples were obtained from 82 patients with EEC who had undergone hysterectomy, and phosphorylated mTOR (p-mTOR) and Akt (p-Akt) expression in the cytoplasm and nucleus was analysed by immunohistochemical staining. Nuclear p-mTOR was significantly elevated in poorly differentiated tumours and positively correlated with lymph node involvement ($P = 0.05$). Nuclear p-mTOR expression was associated with significantly shorter relapse-free survival (RFS) ($P < 0.01$) and slightly shorter overall survival (OS) ($P = 0.08$). Cytoplasmic expression of p-mTOR was not correlated with any clinicopathological factors. Although not significant, cytoplasmic p-mTOR expression was associated with shorter PFS and OS ($P = 0.09$, $P = 0.283$, respectively). Neither cytoplasmic nor nuclear p-Akt expression was associated with clinicopathological factors or with survival. Localisation of p-mTOR may be critical for tumour progression and outcomes in patients with EEC.

1. Introduction

Endometrial cancer is commonly confined to the uterus at diagnosis. The 26th Annual Report of the International Federation of Gynecology and Obstetrics (FIGO) demonstrated that 83% of endometrial cancer patients were stages I and II.[1] As a result, endometrial cancer is considered to be a 'good cancer', as most patients present with early-stage, highly curable disease. Despite the favourable characteristics for most patients, those with high-risk factors, including increased age, higher tumour grade, aggressive histology and advance-stage disease, face challenges.[2]

Current clinical controversies centre on the extent of nodal surgery and the changing roles of radiation therapy and chemotherapy. Recently, findings from the Survival Effect of Para-Aortic Lymphadenectomy (SEPAL) study have shown that pelvic and paraaortic lymphadenectomy can provide survival benefits for patients at intermediate or high risk for recurrence. The results also suggest that adjuvant chemotherapy could further improve survival of patients at high risk of lymph node metastasis.[3] Response rates in the range of approximately 20–30% have been observed with single-agent anthracyclines, platinum compounds and taxanes.[4–8] While combination regimens can achieve higher response rates

* *Corresponding author:* Tel.: +81 776 61 3111; fax: +81 776 61 8111.
E-mail address: yyoshida@u-fukui.ac.jp (Y. Yoshida).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2010.09.004

and improve survival, these therapies are associated with an increased risk of toxicity.[9] Enhanced understanding of biologically relevant targets has fostered the development of new classes of agents that attempt to exploit susceptible pathways in tumour cells.[4]

The phosphatase and tensin homologue (PTEN) mutation is strongly associated with endometrial endometrioid adenocarcinoma (EEC), which is thought to be an oestrogen-dependent tumour and accounts for more than 80% of all cases of endometrial cancer.[10] As PTEN functions as a phosphatase to reduce intracellular lipid products of phosphatidylinositol 3-kinase (PI3K) and block the activation of Akt, inactivation of PTEN results in the activation of Akt, which leads to the activation of mTOR.[4] Therefore, loss of PTEN function could promote the Akt/mTOR signal transduction pathway in EEC.

Akt has been recognised as a key intermediate in signalling pathways that regulate cellular processes involved in cell growth, proliferation, survival and neovasculation.[15] Akt has been shown to play an important role in the chemotherapeutic resistance of tumour cells. The Akt signal transduction pathway is thus considered a promising target for chemotherapy.[11] It can be speculated that decreased PTEN expression with loss of differentiation in EEC could contribute to the emergence of tumours with more aggressive phenotype.[12] Chaudhry and colleagues showed that AKT was significantly phosphorylated in tumour tissue with a loss of PTEN expression, and that phosphorylated-Akt (p-AKT) expression was negatively correlated with PTEN expression.[12] This basic finding supports the notion that AKT activation accompanied by PTEN inactivation is a key step in the development and/or progression of EEC. Uegaki and colleagues reported that loss of PTEN expression was significantly associated with positive p-AKT expression. They also reported a higher survival rate in endometrial cancer patients with PTEN-positive and p-AKT negative expression.[13] However, there have also been several reports that elevated Akt activity is not associated with tumour progression or poor outcomes in ECC.[14–16]

The serine/threonine kinase mammalian target of rapamycin (mTOR) is a central regulator of anabolic processes and cell growth in eukaryotic cells.[17] In mammalian cells, two translational components, ribosomal p70S6 kinase (S6K1) and eukaryotic translation initiation factor-4E (eIF4E) binding protein 1(4E-BP1), are the best characterised downstream effector molecules of mTOR. Increasing evidence has implicated mTOR as a sensor that integrates extracellular and intracellular events, coordinating growth and proliferation.[17] mTOR inhibitors are therefore a potential therapy, particularly in endometrial cancers that exhibit loss of PTEN function. Several mTOR inhibitors (such as temsirolimus, amplimexon and everolimus) have been utilised in a clinical phase II trial for endometrial cancer with modest success.[4] Trials to design combinations of mTOR inhibitors with hormones, antiangiogenic agents and chemotherapy are underway. These approaches are theoretically promising, but combination regimens have been difficult to develop to date because of increased toxicity.[4] The ability to predict which patients derive the most clinical benefit from treatment with mTOR inhibitors would undoubtedly enhance treatment outcome. However, few studies have assessed correlations between mTOR expression in EEC and either clinicopathological features or outcomes.[14–16,18,19]

Both Akt and mTOR are activated by phosphorylation. The present study examined correlations between phosphorylated mTOR (p-mTOR) expression, and clinicopathological features, outcomes and p-Akt expression in EEC. The expression of p-mTOR and p-Akt was evaluated immunohistochemically.

2. Materials and methods

2.1. Patients

The study group included 82 patients with EEC who underwent total abdominal or radical hysterectomy plus bilateral salpingo-oophorectomy with or without lymphadenectomy over a 5-year period at the University of Fukui Hospital (Table 1). Clinicopathological characteristics and follow-up data were obtained from the subjects' medical records. Staging, histology and grading criteria were based on the 1988 FIGO surgical staging classification.[20] Definitive diagnosis was determined by postoperative histopathology, and all specimens were evaluated by subsequent immunohistochemical analysis. Patients with deep myometrial invasion, cervical involvement, lymph vascular space involvement, special histology (such as undifferentiated adenocarcinoma), positive peritoneal cytology or lymph node metastasis were treated with 4–6 rounds of chemotherapy consisting of 180 mg/m^2 paclitaxel and carboplatin according to Chatelut's formula (area under the curve = 5 mg/mL/min) as postoperative adjuvant therapy. All patients were evaluated for disease recurrence by physical examination and a yearly pap smear of

Table 1 – Clinicopathological features of 82 endometrioid endometrial cancers.

		No. (N = 82)	%
Patient age	Median (years)	58.2 ± 12.1	
	Range	38–92	
Clinical stage	I	60	73
	II	10	12
	III	6	7
	IV	6	7
Histological grade	Grade 1	51	62
	Grade 2	19	23
	Grade 3 and undifferentiated	12	15
Myometrial invasion	<50%	60	73
	>50%	22	27
LVSI	Positive	18	25
	Negative	62	76
	Miss	2	2
LN metastases	Positive	5	6
	Negative	62	76
	Miss	15	18
Recurrence	No recurrence	69	84
	Recurrence	13	16

the vaginal vault. Patients were also evaluated by diagnostic imaging (including ultrasonography, computed tomography and magnetic resonance imaging) every 3–6 months with examination of tumour markers. This study was approved by the institutional review board of the University of Fukui Hospital, and written informed consent was obtained from all patients.

2.2. *Immunohistochemistry*

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue by using the avidin–biotin–peroxidase complex technique with a LSAB kit (Dako, Glostrup, Denmark). Sections (2.5 μm) were dewaxed in xylene for 15 × 3 min, rehydrated in an alcohol row, and subjected to antigen retrieval in a pressure cooker for 15 min in 10 mM sodium citrate buffer (pH 6.0). After cooling, they were washed three times in phosphate-buffered saline (PBS, pH 7.2). Endogenous peroxidase activity was blocked by immersion in 3% hydrogen peroxide for 5 min. Non-specific binding of primary antibodies was blocked by incubating sections with diluted normal serum (Dako Protein Block Serum-Free) for 10 min at room temperature. Samples were then incubated with primary antibody to p-Akt (rabbit monoclonal, 3787, 1:50 dilution, Cell Signaling Technology Inc., Beverly, MA, USA) and p-mTOR (Ser^{2448}) (rabbit monoclonal, 49F9, 1:50 dilution, Cell Signaling Technology Inc., Beverly, MA, USA). Sections were incubated overnight with primary antibodies diluted in PBS. After washing with PBS, they were incubated for 10 min with diluted biotinylated goat antimouse immunoglobulins (Dako LSAB kit Bottle 1), used as secondary antibody. After incubation with the avidin-biotin peroxidase complex (Dako LSAB kit Bottle 2) for another 10 min and a repeated washing step with PBS, visualisation was performed with substrate and 3,3′-diaminobenzidine in chromogen solution (Dako EVISION + kit). Sections were then counterstained with Mayer's acidic haematoxylin and washed in an alcohol multiple-row (70–100%). After xylene treatment, sections were covered. Sections from human colon and breast cancers were used as positive controls, and negative controls were obtained by omission of the primary antibody.

The intensity and distribution of the p-Akt and p-mTOR immunohistochemical staining reaction was evaluated using a semi-quantitative method (IRS-score) as described previously.[21] IRS-score was calculated as follows: IRS = ΣSI × PP, where SI is the optical stain intensity (graded as 0 = no, 1 = weak, 2 = moderate, 3 = strong staining). PP is the degree of staining among cells (defined as 0 = no staining, 1 = <10%, 2 = 11–50%, 3 = 51–80%, and 4 = >81%). An IRS-score of more than 6 was defined as 'positive' expression and less than 6

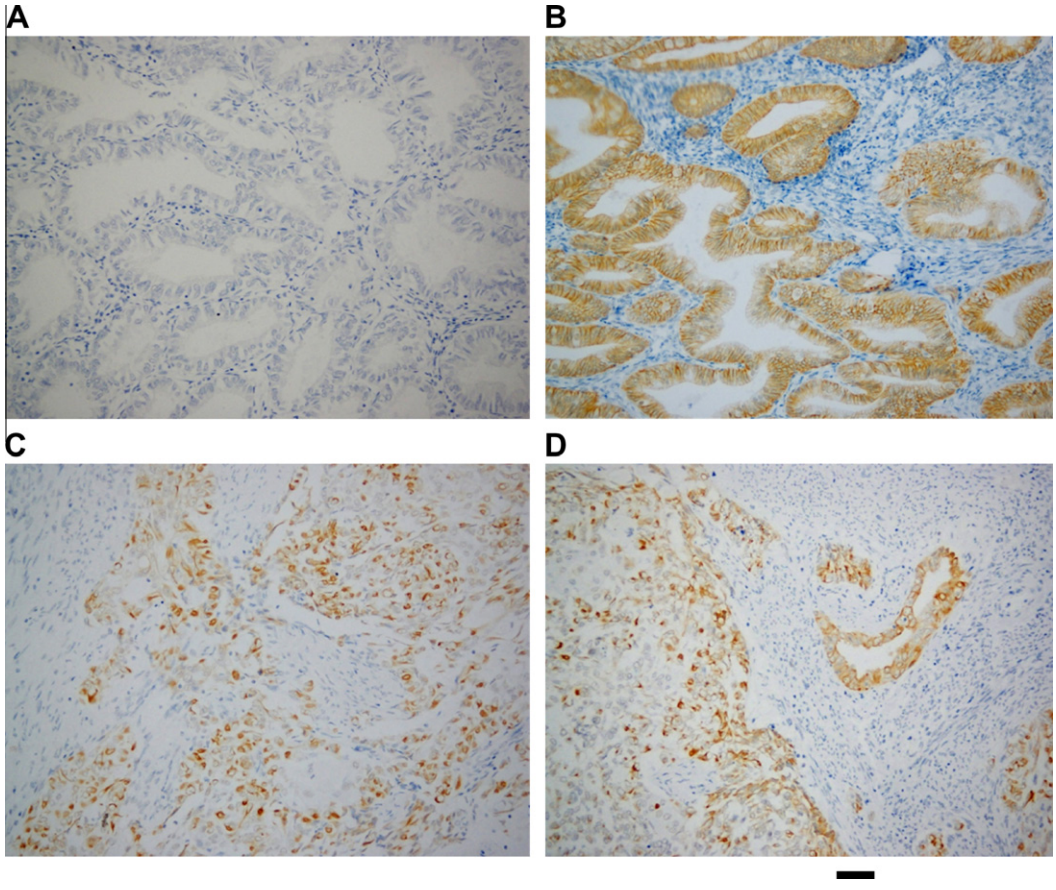

Fig. 1 – Representative endometrioid endometrial cancer showing immunostaining for p-mTOR. Negative for expression of both cytoplasmic and nuclear p-mTOR (A), positive only for expression cytoplasmic p-mTOR (B), positive only for expression of nuclear p-mTOR (C), and positive for expression of both cytoplasmic and nuclear p-mTOR (F) (magnification, ×200).

was defined as 'negative' expression. Immunostaining was scored by two independent observers. Cytoplasmic staining and nuclear staining were evaluated separately.

2.3. Statistical analysis

The χ^2 test was used to test possible associations between the p-Akt or p-mTOR and clinicopathological factors. It was also used to assess correlations between p-Akt and p-mTOR expression. Kaplan–Meier curves were plotted to assess the effects of p-Akt and p-mTOR expressions on relapse-free survival (RFS) and overall survival (OS). Survival curves were compared using the long-rank test. *P*-values $\leqslant 0.05$ were considered statistically significant. Multivariate proportional Cox models were used to assess the prognostic significance of p-Akt and p-mTOR expression and of several clinicopathological factors. Statistical analysis was performed using a software package (SPSS for Windows 14.0, SPSS Inc., Chicago, IL, USA).

3. Results

Expression of p-mTOR was seen in both the cytoplasm and nuclei of EEC cells (41% and 21%, respectively) (Fig. 1), as was expression of p-Akt (82% and 7%, respectively). There was a significantly positive correlation between nuclear and cytoplasmic expression of p-mTOR ($P = 0.02$), but there was no correlation between nuclear p-Akt and cytoplasmic p-Akt expression, nor was there a correlation between p-mTOR and p-Akt expression in either the nucleus or cytoplasm (Table 2A). Nuclear p-mTOR expression was positively correlated with histological grade ($P = 0.002$) and marginally positively correlated with lymph node involvement ($P = 0.05$). Rates of nuclear p-mTOR expression were higher among advanced-stage and deep myometrial invasion groups, but the difference was not significant. Cytoplasmic expression of p-mTOR was not correlated with any clinicopathological factors, and there were no correlations between p-Akt expression and clinicopathological factors (Table 2B).

Patients positive for nuclear p-mTOR expression had significantly shorter PFS ($P < 0.01$), and had a tendency for shorter OS ($P = 0.08$) than those negative for nuclear p-mTOR expression (Fig. 2A). Although not significant, patients positive for cytoplasmic p-mTOR expression had shorter PFS (Fig. 2B) and OS than those negative for cytoplasmic p-mTOR expression ($P = 0.09$, $P = 0.283$, respectively). In contrast, nuclear and cytoplasmic p-Akt expression was not associated with shorter PFS ($P = 0.902$, $P = 0.605$, respectively) (Fig. 3A and B) or OS ($P = 0.578$, $P = 0.933$, respectively). Patients were classified into the following four subgroups according to p-mTOR expression and PFS survival was analysed: patients negative for expression of both cytoplasmic and nuclear p-mTOR (group A), patients positive only for expression of cytoplasmic p-mTOR (group B), patients positive only for expression of nuclear p-mTOR (group C), and patients positive for expression of both cytoplasmic and nuclear p-mTOR (group D) (Fig. 4). In patients positive only for expression of nuclear p-mTOR, RFS was the shortest among the four groups ($P < 0.01$). In patients positive only for expression cytoplasmic p-mTOR, RFS was found to be significantly favourable among all four groups.

The prognostic relevance of m-TOR and p-Akt expression was analysed using a multivariate proportional-hazards model adjusted for established clinical prognostic factors (i.e. depth of tumour invasion, lymph node involvement, histological grade, stage) (Table 3). Histological grade was an independent prognostic factor for RFS (hazard ratio (HR) = 3.56, 95% confidence interval (CI) 2.625–475.101, $P = 0.007$), but expression of nuclear p-mTOR was not independent (HR = 0.12, 95% CI 0.079–9.984, $P = 0.159$). In addition, there were no correlations between nuclear p-mTOR expression and recurrence site.

4. Discussion

The present study indicated that the distribution of mTOR is critical for outcomes of EEC. It is widely accepted that mTOR is predominantly localised in the cytoplasm, and is associated with a variety of intracellular membrane structures.[22–24] However, mTOR shuttles between the nucleus and cytoplasm; a small fraction of mTOR is seen in the nucleus.[25,26] As the present study indicated, in patients with EEC, the mechanism of mTOR import into the nucleus from the cytoplasm may play an important role in tumour grade and outcomes.mTOR exerts multiple functions in the context of two different multi-protein complexes; mTOR complex 1 (mTORC1) contains

Table 2A – Correlations between nuclear and cytoplasmic expression of p-mTOR and p-Akt.

	p-mTOR (nuclear)			p-Akt (cytoplasmic)			p-Akt (nuclear)		
	Negative	Positive	*P*-value	Negative	Positive	*P*-value	Negative	Positive	*P*-value
P-mTOR (cytoplasmic)									
Negative	42	23	0.029*	11	37	0.35	43	5	0.2
Positive	6	11		5	29		33	1	
P-mTOR (nuclear)									
Negative				12	55	0.69	60	5	0.79
Positive				4	13		16	1	
P-Akt (cytoplasmic)									
Negative							16	0	0.21
Positive							60	6	

* $P < 0.05$.

Table 2B – Correlations between p-mTOR expression or p-Akt and clinicopathological factors.

	p-Akt (nuclear)			p-Akt (cytoplasmic)			p-mTOR (nuclear)			p-mTOR (cytoplasmic)		
	Negative	Positive	P-value Negative	Negative	Positive	P-value	Negative	Positive	P-value	Negative	Positive	P-value
Stage												
Negative	65	5	0.88	12	58	0.19	58	7	0.06	43	5	0.22
Positive	11	1		4	8		12	5		27	7	
Histological grade												
Negative	65	5	0.88	12	58	0.19	60	5	0.002*	42	6	0.54
Positive	11	1		4	8		10	7		28	6	
Myometrial invasion												
Negative	55	5	0.55	10	50	0.28	51	14	0.06	35	13	0.95
Positive	21	1		6	16		9	8		25	9	
Lymph node metastases												
Negative	69	3	0.12	15	57	0.25	59	2	0.05+	42	2	0.64
Positive	4	1	0	5	13	3	30	3				
Lymph vascular space involvement												
Negative	58	4	0.92	10	52	0.28	52	10	0.27	36	8	1.0
Positive	13	1	4	10	10	4	26	6				
Recurrence												
Negative	12	1	0.95	2	11	0.68	7	58	0.02*	6	42	0.36
Positive	64	5	14	55	6	11	7	27				

* $P < 0.05$.
+ $P = 0.05$.

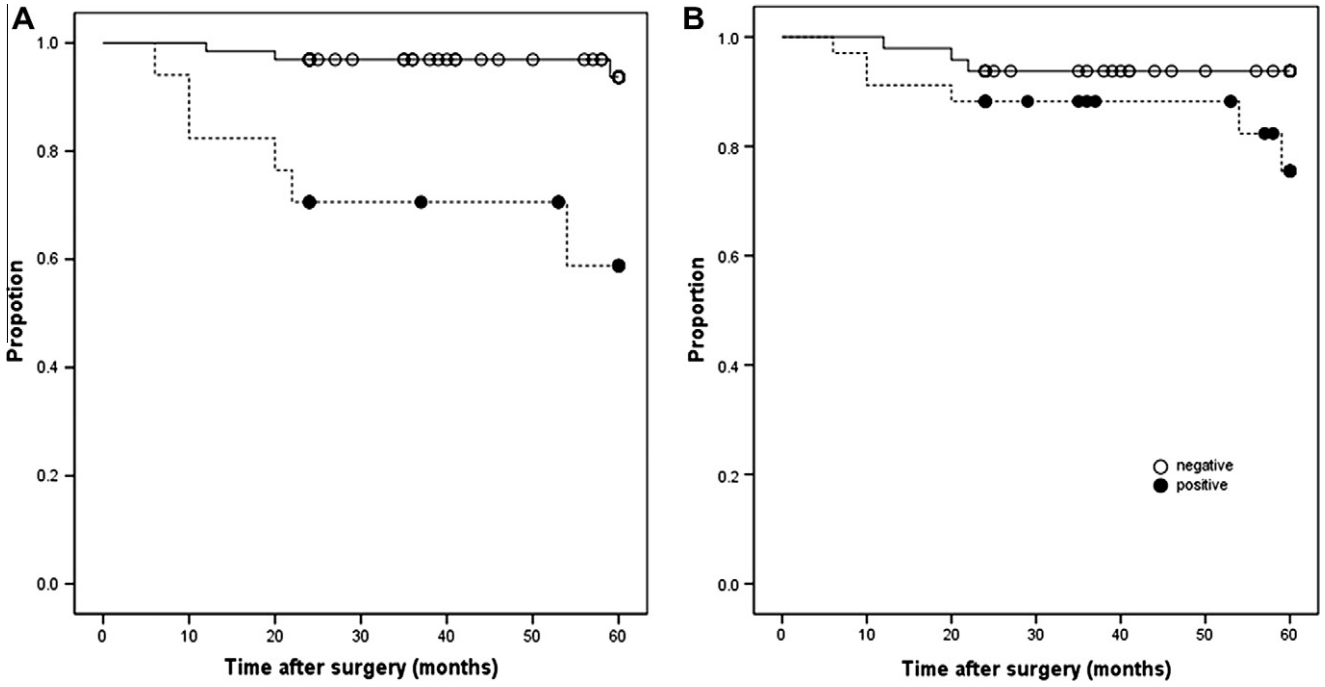

Fig. 2 – Kaplan-Meier curves for relapse-free survival of patients with expression of nuclear p-mTOR expression (A), and cytoplasmic p-mTOR expression in endometrioid endometrial cancer.

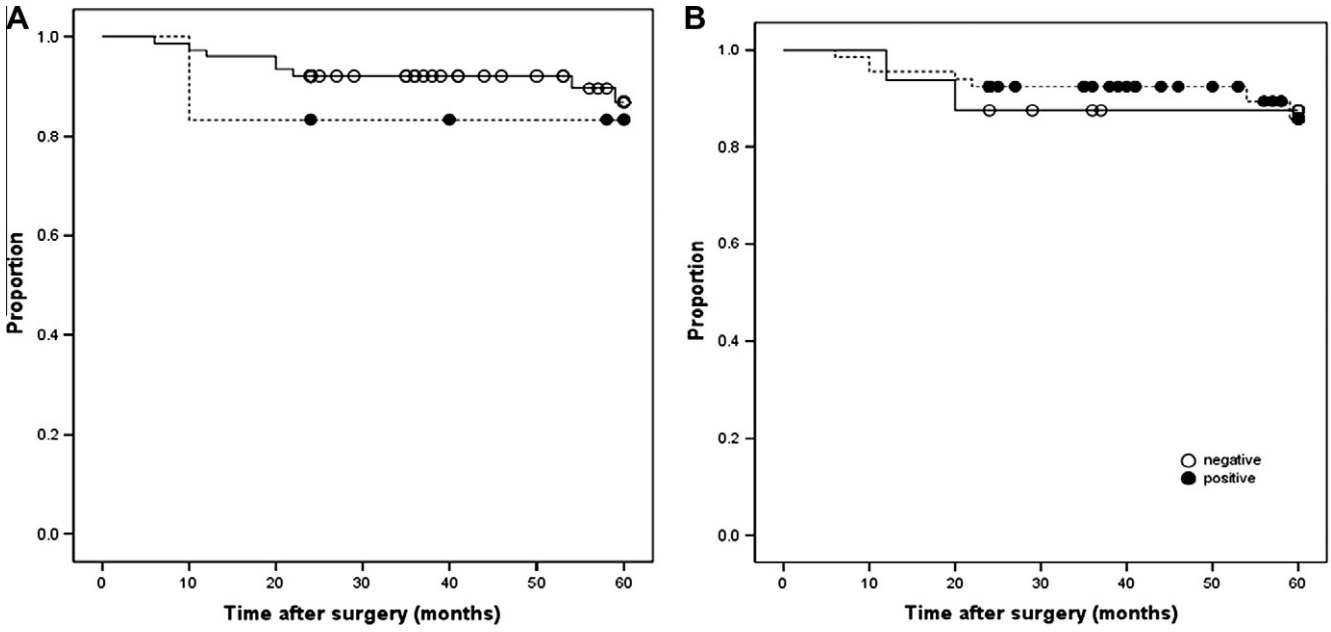

Fig. 3 – Kaplan–Meier curves for relapse-free survival of patients with expression of nuclear p-Akt expression (A), and cytoplasmic p-Akt expression (B) in endometrioid endometrial cancer.

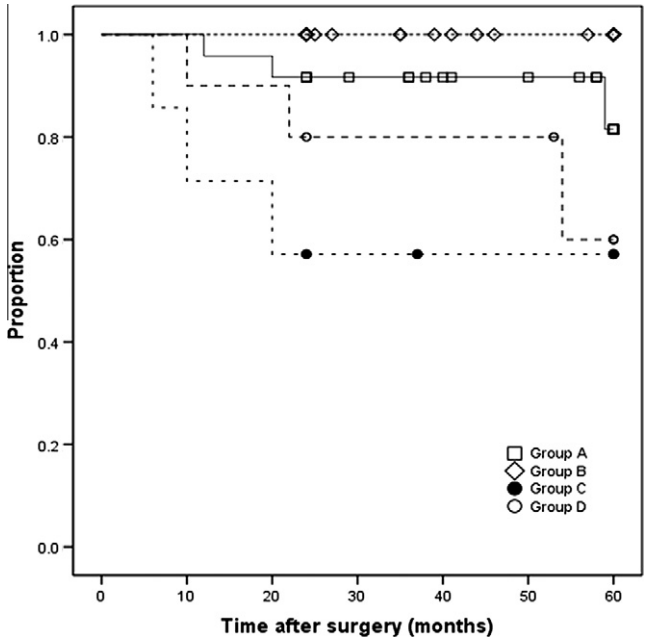

Fig. 4 – Kaplan–Meier curves for relapse-free survival of patients in four subgroups classified according to p-mTOR expression; negative for both cytoplasmic and nuclear p-mTOR expression (group A), positive for only cytoplasmic p-mTOR expression (group B), positive for expressions only nuclear p-mTOR expression (group C), positive for both cytoplasmic and nuclear p-mTOR expression (group D) in endometrioid endometrial cancer.

Table 3 – Prognostic factors in multivariate Cox proportional-hazards regression model.

	HR	95% CI	P-value
Clinical stage	1.17	0.477–22.121	0.229
Histological grade	3.56	2.625–475.101	0.007*
Myometrial invasion	1.02	0.306–25.269	0.364
Lymph node metastases	0.43	0.49–48.119	0.806
Lymph vascular space involvement	0.25	0.248–6.568	0.769
Nuclear p-mTOR	0.12	0.079–9.9838	0.159
Cytoplasmic p-mTOR	2.45	11.623–0.932	0.322
Nuclear p-Akt	1.42	4.133–0.574	0.923
Cytoplasmic p-Akt	0.91	2.494–0.409	0.057

HR, hazard ratio; CI, confidence interval.
* $P < 0.05$.

raptor and mammalian orthologue of yeast Lst8p (mLST8; also known as GβL), activates ribosomal p70S6 kinase (S6K1), and inactivates the regulators of translation eukaryotic translation initiation factor-4E binding protein 1 (4E-BP1), while mTOR complex 2 (mTORC2) contains rictor, mLST8, and sin1 (also known as mitogen-activated protein-kinase-associated protein 1), and is required for Akt phosphorylation on Ser473 to achieve full activation.[22–24]

The molecular mechanisms that regulate mTORC1 kinase activity are still poorly understood, but it is increasingly clear that many, if not most, cancer-promoting lesions activate the mTORC1 pathway. The mTORC1 pathway regulates growth through downstream effectors, such as the regulators of translation 4E-BP1 and p70 S6K1, and promotes translation initiation by phosphorylating those two targets. Activated mTORC1 phosphorylates 4E-BP1. Hypophosphorylated 4EBP1 binds to and thereby inactivates the cap-binding protein eukaryotic translation initiation factor 4E (eiF4E), while after phosphorylation by mTORC1, 4EBP1 releases eiF4E and allows its binding to the cap-structure of mRNA and the beginning of protein translation.[27] 4E-BP1 and S6K1 have been detected exclusively in the cytoplasm of cancer cells.[25] However, in

an immunohistochemical study of endometrial cancer by Darb-Esfahani and colleagues, most cases displayed a distinct nuclear p-4E-BP1 expression of varying intensity. Nuclear p-4E-BP1 expression was reported to be elevated in poorly differentiated cancers.[18] Furthermore, Rojo and colleagues found that the results of breast cancer were comparable to those observed in ovarian tumours, in which p-4EBP1 expression was also associated with high histologic grade and poor survival.[28] These data support the results of the present study: an increase in phosphorylated nuclear mTORC1 complexes may activate signalling to nuclear p-4E-BP1 and contribute to tumour progression.

It was has been reported that mTORC1 exists in the cytoplasm of fibroblast cells, although its distribution in cancer cells is not yet clear. It has been found that the nuclear shuttling of mTOR is critical for signalling its cytoplasmic targets S6K1 and 4E-BP1.[25,26] However, the mechanism of nuclear transportation of mTOR and function of nuclear mTOR remain unclear. It has been assumed that mTOR has a distinct function in the nucleus, and that it undergoes shuttling to coordinate its role in the cytoplasm and nucleus. Recently, Bechmann and colleagues suggested that the existence of a nuclear shuttling signal for mTOR could provide definitive evidence for the requirement of mTOR nuclear import in cytoplasmic signalling with S6K1.[26] Although no previous studies have assessed the expression of S6K1 in ECC, which is also present in oestrogen receptor (ER)-positive breast cancer cells, Yamnik and colleagues found that overexpression of S6K1 regulated ER through phosphorylation of ER-α leads to transcriptional activation of ER, and might be associated with poor prognosis.[29]

While mTORC2 is required for Akt phosphorylation on Ser473 to achieve full activation, it is less well understood than mTORC1. Recent work indicates that it should be considered part of the PI3K-Akt pathway, as it directly phosphorylates Akt on one of the two sites that are necessary for Akt activation in response to growth-factor signalling.[27] Activated Akt was not related to either tumour progression or outcome in this study. Moreover, Mori and colleagues observed no correlation between p-AKT and downstream targets, including p-mTOR in EEC.[14] Therefore, mTORC2 might not be related to these variables.

The present immunohistochemical study indicated that the expression of nuclear p-mTOR is correlated with poor outcomes in EEC. Although 4E-BP1 and S6K1 were not investigated in this study, increased nuclear p-mTOR could be coordinated with phosphorylated 4EBP1 in nuclear and phosphorylating S6K1 in the cytoplasm, and nuclear p-mTOR may contribute to tumour progression and outcomes in EEC.

In the present study, both cytoplasmic and nuclear expression of p-mTOR was observed in EEC (41% and 21% of cases, respectively). The rate of positive p-mTOR in ECC was similar to that seen in the study of Darb-Esfahani; they indicated p-mTOR overexpression was detected in 33.3% of patients and was more frequent in tumours extending outside the uterine corpus.[18] Wahl and colleagues reported that p-mTOR was strongly detected only in the cytoplasm in more than 70% of patients. In addition, they reported that cytoplasmic p-mTOR was expressed in both type I and II endometrial cancers (82.9% and 76.7%, respectively), as well as in benign endometrium.[16] In other words, they indicated that cytoplasmic p-mTOR was detected endometrial cancer as well as normal endometrium. Mori and colleagues also described p-mTOR expression in 92% of endometrial cancer patients (11% had weak expression, 62% had moderate expression, and 19% had strong expression) but reported no assessment of p-mTOR expression as it correlated to clinicopathological features.[14] There have been no previous reports on the different localisation of mTOR expression associated with different outcomes in patients with endometrial cancer. Previously there has only been one report of assessment of nuclear p-mTOR expression. In patients with gastric cancer, Murayama and colleagues showed that different localisation of mTOR expression was associated with different outcomes. They found that cytoplasmic expression of p-mTOR positively was correlated with factors related to tumour progression and poor outcomes, whereas nuclear expression of p-mTOR was negatively correlated with such factors.[30] These discrepancies may be partly explained by differences in the type of cancer studied, in the expression of downstream targets of p-mTOR, and particularly in ER status and signalling.

Several inhibitors of mTOR kinase have been evaluated in endometrial cancer, and their anticancer efficacy has been shown.[4] As mTOR is mainly present in the cytoplasm, tumours with cytoplasmic expression of mTOR may be more sensitive to mTOR inhibitors than those with nuclear expression of mTOR. In addition, nuclear p-mTOR expression was closely related with outcome, thus suggesting that inhibition of mTOR nuclear transport signals is involved with preventing the progression of tumours in EEC.

In conclusion, nuclear p-mTOR expression was associated with tumour progression and poor survival in EEC; there was a significantly poor survival in EEC patients with only nuclear p-mTOR expression, as compared to only cytoplasmic p-mTOR expression. Localisation of p-mTOR might thus be critical to tumour progression and outcomes in patients with EEC. Further investigation is needed to clarify which patients exhibit nuclear transport signals involving mTOR, and for which tumours mTOR kinase inhibitors could be an effective therapy.

Conflict of interest statement

None declared.

Acknowledgements

We would like to thank the staff of the Obstetrics and Gynecology Department at the University of Fukui Hospital, and the many people who assisted in the study. This study was partly funded by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (21592124). We declare the study sponsors had no involvement, such as, in the study design, in the collection, analysis and interpretation of data.

REFERENCES

1. Creasman WT, Odicino F, Maisonneuve P, et al. Carcinoma of the uterus uteri. *Int J Gynecol Oncol* 2006;**95**(1):S105–43.

2. Mcmeenkin DS, Alektiar KM, Sabbatini PJ, Zaino RJ. Corpus: epithelial tumors. In: Barakat RR, Markman M, Randall ME, editors. *Principles and practice of gynecologic oncology.* 5th ed. Baltimore: Lippincott Williams & Wolters Kluwere; 2009. p. 683–733.
3. Todo Y, Kato H, Kaneuchi M, et al. Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. *Lancet* 2010;**375**:1165–72.
4. Ray M, Fleming G. Management of advanced-stage and recurrent endometrial cancer. *Semin Oncol* 2009;**36**:145–54.
5. Thigpen JT, Blessing JA, DiSaia PJ, et al. A randomized comparison of doxorubicin alone versus doxorubicin plus cyclophosphamide in the management of advanced or recurrent endometrial carcinoma: A Gynecologic Oncology Group study. *J Clin Oncol* 1994;**12**:1408–14.
6. Calero F, Asins-Codoner E, Jimeno J, et al. Epirubicin in advanced endometrial adenocarcinoma: a phase II study of the Grupo Ginecologico Espanol para el Tratamiento Oncologico (GGETO). *Eur J Cancer* 1991;**27**:864–6.
7. van Wijk FH, Lhomme C, Bolis G, et al. Phase II study of carboplatin in patients with advanced or recurrent endometrial carcinoma. A trial of the EORTC Gynaecological Cancer Group. *Eur J Cancer* 2003;**39**:78–85.
8. Lissoni A, Zanetta G, Losa G, et al. Phase II study of paclitaxel as salvage treatment in advanced endometrial cancer. *Ann Oncol* 1996;**7**:861–3.
9. Fleming GF, Brunetto VL, Cella D, et al. Phase III trial of doxorubicin plus cisplatin with or without paclitaxel plus filgrastim in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. *J Clin Oncol* 2004;**22**:2159–66.
10. Risinger JI, Hayes K, Maxwell GL, et al. PTEN mutation in endometrial cancers is associated with favorable clinical and pathologic characteristics. *Clin Cancer Res* 1998;**4**:3005–10.
11. Altomare DA, Testa JR. Pertubations of the AKT signaling pathway in human cancer. *Oncogene* 2005;**24**:7455–64.
12. Chaudhry P, Asselin E. Resistence to chemotherapy and hormone therapy in endometrial cancer. *Endocrine-Related Cancer* 2009;**16**:363–80.
13. Uegaki K, Kanamori Y, Kigawa J, et al. PTEN-positive and phosphorylated-Akt-negative expression is a predictor of survival for patients with advanced endometrial carcinoma. *Oncol Rep* 2005;**14**:389–92.
14. Mori N, Kyo S, Sakaguchi J, et al. Concomitant activation of AKT with extracellular-regulated kinase 1/2 occurs independently of PTEN or PIK3CA mutations in endometrial cancer and may be associated with favorable prognosis. *Cancer Sci* 2007;**98**:1881–8.
15. Steinbakk A, Skaland I, Gudlaugsson E, et al. The prognostic value of molecular biomarkers in tissue removed by curettage from FIGO stage 1 and 2 endometrioid type endometrial cancer. *Am J Obstet Gynecol* 2009;**200**:78.e1–8.
16. Wahl H, Daudi S, Kshirsagar M, et al. Expression of metabolically targeted biomarkers in endometrial carcinoma. *Gynecol Oncol* 2010;**116**:21–7.
17. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006;**124**:471–84.
18. Darb-Esfahani S, Faggad A, Noske A, et al. Phospho-mTOR and phospho-4EBP1 in endometrial adenocarcinoma: association with stage and grade in vivo and link with response to rapamycin treatment in vitro. *J Cancer Res Clin Oncol* 2009;**135**:933–41.
19. No JH, Jeon Y-H, Park I-A, et al. Expression of mTOR protein and its clinical significance in endometrial cancer. *Med Sci Monit* 2009;**15**:301–5.
20. FIGO stages – 1988 revision. *Gynecol Oncol* 1989;35:125–7.
21. Nur S, Chuang L, Ramaswamy G. Immunohistochemical characterization of cancer antigen in uterine cancers. *Int J Gynecol Cancer* 2006;**16**:1903–10.
22. Sabatini DM, Barrow RK, Blackshaw S, et al. Interaction of RAFT1 with gephyrin required for rapamycin-sensitive signaling. *Science* 1999;**284**:1161–4.
23. Tirado OM, Mateo-Lozano S, Sanders S, Dettin LE, Notario V. The PCPH oncoprotein antagonizes the proapoptotic role of the mammalian target of rapamycin in the response of normal fibroblasts to ionizing radiation. *Cancer Res* 2003;**63**:6290–8.
24. Drenan RM, Liu X, Bertram PG, Zheng XF. FKBP12-rapamycin-associated protein or mammalian target of rapamycin (FRAP/mTOR) localization in the endoplasmic reticulum and the Golgi apparatus. *J Biol Chem* 2004;**279**:772–8.
25. Zhang X, Shu L, Hosoi H, Murti KG, Houghton PJ. Predominant nuclear localization of mammalian target of rapamycin in normal and malignant cells in culture. *J Biol Chem* 2002;**277**:28127–34.
26. Bachmann RA, Kim JH, Wu AL, Park IH, Chen J. A nuclear transport signal in mammalian target of rapamycin is critical for its cytoplasmic signaling to S6 kinase 1. *J Biol Chem* 2006;**281**:7357–63.
27. Sabatini DM. MTOR and cancer: insights into a complex relationship. *Nat Rev Cancer* 2006;**6**:729–34.
28. Rojo F, Najera L, Lirola J, et al. 4E-binding protein 1, a cell signaling hallmark in breast cancer that correlates with pathologic grade and prognosis. *Clin Cancer Res* 2007;**13**:81–9.
29. Yamnik RL, Digilova A, Davis DC, et al. S6 kinase 1 regulates estrogen receptor alpha in control of breast cancer cell proliferation. *J Biol Chem* 2009;**284**:6361–9.
30. Murayama T, Inokuchi M, Takagi Y, et al. Relation between outcomes and localisation of p-mTOR expression in gastric cancer. *Br J Cancer* 2009;**100**:782–8.