Expression of the mTOR Pathway Regulators in Human Pituitary Adenomas Indicates the Clinical Course

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Abstract. *Pituitary ademonas are benign tumours with different biological behaviour, especially with regard to tumour size, invasion, endocrine function, intratumour cystic lesion and apoplexy. There is little understanding of the growth and the control of progression of pituitary tumours. In the present study, we investigated the expression of mammalian target of rapamycin (mTOR) pathway regulators, in clinical pituitary adenomas. Pituitary adenomas from 95 patients were included in the study. Fresh pituitary tumours were obtained immediately after surgery and processed for histological, immunohistological and molecular based analyses. Histolopathological and clinical information including tumour stage, invasion characteristic and endocrine status were analysed against the gene transcript expression of mTOR, RAPTOR and RICTOR. There was a stepwise and significantly increased relation-ship between RICTOR expression and tumour size, namely p=0.0012 and p=0.0055 for tumours 1-2 cm and tumours >3 cm compared with tumours <1 cm respectively. Significantly higher levels of mTOR were seen in tumours with cystic lesions (p=0.044). There was no significant correlation between mTOR, RAPTOR and RICTOR and tumour apoplexy, nor a correlation between mTOR, RAPTOR and RICTOR with suprasephanous spread and sella floor destruction. However, pituitary tumours with cavernous sinus invasion, namely Knosp stage 3-4 had significantly lower levels of RAPTOR than those of Knosp stage 1-2 (p=0.01). A similar but statistically insignificant trend was seen with RICTOR. Using modified Hardy's staging, it was found that there was a significant correlation between tumour stage and RAPTOR and RICTOR expression. mTOR and RAPTOR levels differed in tumours with different endocrine functions, although no statistical difference was observed. However, Growth Hormone (GH) -, Follicle-Stimulating Hormone (FSH)-, Thyroid Stimulating Hormone (TSH)-secreting tumours had significantly lower levels of RICTOR compared with nonfunctional tumours. Finally, levels of mTOR were found to be significantly correlated with levels of both RAPTOR and RICTOR. It is noteworthy that RAPTOR and RICTOR levels were also significantly correlated. In conclusion, mTOR pathway regulators, mTOR, RAPTOR and RICTOR are significantly correlated with the invasion, staging, and tumour growth of pituitary adenomas and thus have an important predictive and prognostic value in patients with pituitary adenoma.*

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Key Words: Pituitary adenoma, mTOR, RAPTOR, RICTOR, staging, invasion.

Mammalian target of rapamycin (mTOR) (also known as the mechanistic target of rapamycin) is a serine/threonine kinase that belongs to the Phosphatidylinositide 3-kinases (PI 3-kinases or PI3Ks) family (1). It is a key intracellular signalling regulator that connects the extracellular events to the intracellular process. A protein of about 289 kDa, mTOR forms two major protein complexes with other protein partners, namely mTORC1 and mTORC2, which differ in the protein components and in their sensitivity to rapamycin. mTORC1 is formed by mTOR, Regulatory-associated protein of mTOR (RAPTOR), Proline-roch Akt Substrate 40 KDa (PRAS40), and DEP domain containing mammalian target of rapamycin (mTOR) (DEPTOR). mTORC2 is formed by mTOR, Rapamycin Independent Companion of mTOR (RICTOR), DEPTOR, stress-activated protein kinase-interacting protein 1 (SIN1) and Protor-I (2-4). A major difference between the two complexes is the mTOR regulating proteins RAPTOR and RICTOR. Interactions between mTOR and RAPTOR and RICTOR are mutually exclusive. RICTOR, is essential in recruiting other mTORC2 proteins to the protein complex. A similar role for RAPTOR (also called KIAA1303) is known for the mTORC1 complex (5-7).

For the mTORC1 complex, extracellular signals including cytokines, inflammatory intermidators, oxygen and energy levels send signals to AKT and the Tuberous Sclerosis Complex (TSC)1/2 proteins, negative regulators of mTOR, which deactivate small Ras-related GTPase (8-10). This results in activation of mTORC1 and its downstream pathways including S6 Kinase-1 (S6K1), PRAS40 and Lipin1, and regulate protein synthesis, suppression of autophagy, gene expression, mitochondrial metabolism and adipogenesis metabolisms. On the other hand, activation of mTORC2 will either go on to activate the mTORC1 pathway, or directly regulate the PKC, ion transportation and cell growth *via* serum- and glucocorticoid-regulated kinase (SGK) pathways (11,12). There is compelling evidence which show that the mTOR pathways are aberrant in cancer, whether *via* over expression of the mTOR pathway molecules, loss of mutation of mTOR negators such as TSC proteins, or excessive extracellular signals such as cytokines. Collectively, this has made mTOR pathways viable targets in cancer treatment.

Pituitary adenomas are benign tumours with monoclonal origin. Pituitary adenomas have overall prevalence of 16.7% in the general population, 14.4% in autopsy studies and 22.5% in radiological studies (13). Although most tumours are slow growing, they do however have aggressive clinical course, including invasion of surrounding tissues, namely sella bones *etc*.

The mTOR pathway takes part in the process of tumour development and may have a role in the development and progression of pituitary tumours. Dai *et al*. affirmed that down regulation of PI3K/AKT/mTOR pathway improved the chemotherapy result in pituitary adenoma cell lines *in vitro* and in xenografted pituitary adenoma in nude mice (14). Some of the tumours showed a rapid rate of growth, and invaded the suprasella region, sphenoid sinus and cavernous sinus. Clinically, it is very difficult for neurosurgeons to totally remove pituitary tumours, which can reoccur rapidly after initial surgery. Invasive pituitary adenomas are obviously different from the classical ones. There may be a relationship between mTOR pathways and unrestrained growth of invasive pituitary adenoma. Although there was no difference found in phosphorylated mTOR in pituitary adenomas and control tissues, there are indications that its regulators may be activated and that there may be possible changes in message expression of mTOR (15). In pituitary adenomas which secrete growth hormone and in non-functional pituitary adenomas, mTOR has been found to be active (16, 17). In non-human pituitary adenoma cells, PI3K/mTOR inhibitor, XL765, was shown to influence the growth and apoptosis of tumour cells (14). Inhibiting mTOR pathway also reduced the growth of pituitary adenoma cells (18, 19). RAPTOR and RICTOR are mutually exclusive proteins when forming the mTOR complex, mTORC1 and mTORC2. The two mTOR complexes have related but also different functions in cells. It is unclear if mTOR, RAPTOR and RICTOR have a related expression pattern and if they play a distinct or orchestrated role (s) in cancer. In a recent report, it was shown that these three molecules were aberrantly expressed in human breast cancer (20). In the present study, we investigated the expression of mTOR, RAPTOR and RICTOR in human pituitary adenomas and aimed to establish a possible link between the expression pattern, and their relationship with the pathological and clinical features of pituitary adenomas.

Table I. *Clinical and pathological information of the patients*.

Group		n
Gender	Male	51
	Female	44
Age, years	<45	45
	>45	50
Tumour size, cm	<1	2
	1-2	17
	2-3	34
	>3	42
Intratumoral haemorrhage	No	77
	Yes	18
Suprasella invasion	No	59
	Yes	36
Knosp-grade	0-2	70
	3-4	25
Invasion	No	68
	Yes	27
Hardy's stage		
	I	9
	II	32
	III	26
	IV	28
Endocrine tumour	No	59
	PRL	5
	GH	8
	FSH	2
	ACTH	6
	TSH	4
	LH	2
	Mixture	9

PRL: Prolactin; GH: growth hormone; FSH: Follicle-Stimulating Hormone; ACTH: adreno-cortico-tropic-hormone; TSH: Thyroid Stimulating Hormone; LH: luteinizing hormone.

Materials and Methods

Clinical and pathological demographics of the patients. Patient's clinical history, diagnostic images, endocrine tests were routinely recorded. This study included a total of 95 patients with pituitary adenomas who underwent transsphenoidal or craniotomy surgical resection from Jan 2012 to Dec 2012 at the Department of Neurosurgery of Beijing TianTan Hospital, PRC. Age, gender,

Table II. *Primers used in the study.*

	5'-'3	5'-'3
Mammalian Target Of Rapamycin (mTOR)	gctgcagaagaaggtcact	actgaacctgaccgtacaaaggagatggaacggaag
Regulatory associated protein of mTOR (RAPTOR)	tgaacaccggaccatgac	actgaacctgaccgtacacaatgaggtttccctgaag
Rapamycin Independent Companion of mTOR (RICTOR)	aacttgcaaaacagtgtgaa	actgaacctgaccgtacaatatcacagccttgtttggt
glyceraldehyde-3-phosphate dehydrogenas GAPDH	ctgagtacgtcgtggagtc	actgaacctgaccgtacacagagatgatgaccctttg

Table III. *Expression level of Mammalian Target Of Rapamycin (mTOR), Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR* (*RICTOR*) *according to clinicopathological characteristices of patient.*

		mTOR	RAPTOR	RICTOR
Gender	Male	5.6±2.57	3.3±1.04	0.674±0.166
	Female	2.527±0.415	3.68±1.49	1.263±0.307
Age, years	<45	4.29±1.33	5.35±1.71	0.995±0.285
	>45	4.07±2.38	1.793±0.584	0.904±0.198
Tumour size,cm	<1	1.444±0.431	0±0	0.0799±0.046
	1-2	1.811±0.423	2.1±0.829	0.573±0.124
	>3	4.24±1.42	3.553±0.899	0.965±0.173
Cystic lesions	No	3.27±0.824	5.01±1.6	1.127±0.269
	Yes	5.14±2.75	1.841±0.568	0.755±0.201
Introtumour Haemorrhage	No	3.207±0.786	3.195±0.975	1.002±0.194
	Yes	8.33±6.61	4.69±2.1	0.711±0.34
Supsella Spread	No	2.689±0.811	3.11±1.06	0.776±0.147
	Yes	6.62±3.42	4.09±1.57	1.226±0.376
Cavernous sinus invasion staging	Knosp 1-2	3.355±0.894	4.12±1.21	1.082±0.233
	Knosp 3-4	6.14±4.25	1.946±0.683	0.623±0.131
Sella floor destruction	No	3.259±0.992	4.13±1.25	1.055±0.229
	Yes	6.51±4.09	2.85±1.27	0.858±0.285
Invasion	No	3.68±1.24	4.41±1.62	0.993±0.229
	Yes	4.66±2.49	2.561±0.728	0.901±0.252
Hardy's stage	I	1.444±0.431	0±0	0.0799±0.046
	II	3.17±1.33	3.6±1.67	0.781±0.17
	III	3.91±1.59	6.17±2.28	1.446±0.514
	IV	5.56±3.5	1.655±0.643	0.816±0.246
	I&II	3.08±1.26	3.4±1.58	0.743±0.163
	III&IV	4.88±2.14	3.52±1.05	1.077±0.257
Endocrine	Nonfunctionnal	5.27±2.22	3.65±1.27	1.136±0.257
	Functionnal	2.383±0.534	3.2±1.08	0.637±0.142
	PRL	3.78±2.46	4.31±2.41	0.532±0.281
	GH	1.97±1.16	4.09±2.4	0.1271±0.0416
	FSH	1.5655±0.0377	1.37±1.37	0.485±0.176
	ACTH	0.959±0.45	1.152±0.971	0.841±0.634
	TSH	4.28±2.12	8.92±7.64	0.512±0.147
	LH	5.03±3.03	0.894±0.727	1.246±0.375
	Mixed	1.668±0.609	1.52±1.05	0.967±0.305

PRL: Prolactin; GH: growth hormone; FSH: Follicle-Stimulating Hormone; ACTH: adreno-cortico-tropic-hormone; TSH: Thyroid Stimulating Hormone; LH: luteinizing hormone.

hormonal function were also reviewed and recorded. The diagnosis was confirmed by postoperative pathology. Immunohistochemical examination was used to determine the endocrine type. Preoperative magnetic resonance imaging (MRI) was performed to determine image characteristic: tumour size, cystic lesion, intratumour haemorrhage, invasion type. The greatest diameter of tumour obtained on gadolinium (Gd)-enhanced T1WI MRI was measured as the tumour size. Intratumoral haemorrhage and cystic lesion were defined by preoperative MRI and were confirmed intraoperatively. The invasion type was determined based on the invasion site of the tumour and recorded as cavernous sinus invasion, sphenoid sinus invasion, and suprasellar invasion. The criteria for suprasellar

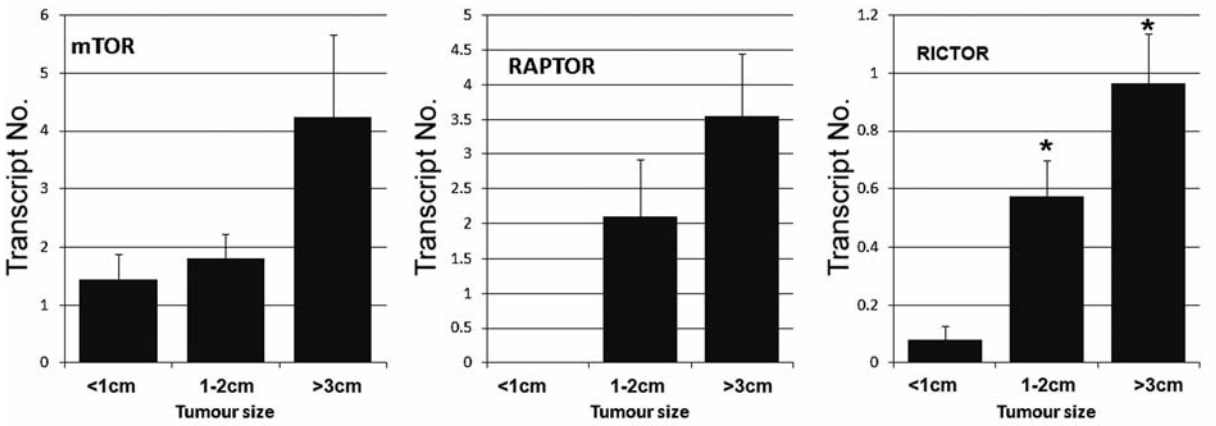

Figure 1. *Levels of Mammalian Target Of Rapamycin (mTOR), Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR (RICTOR) are correlated with the tumour size in pituitary adenomas. *The level of the RICTOR transcripts for tumours 1-2 cm and tumours >3 cm are significantly higher than that of tumours <1 cm.*

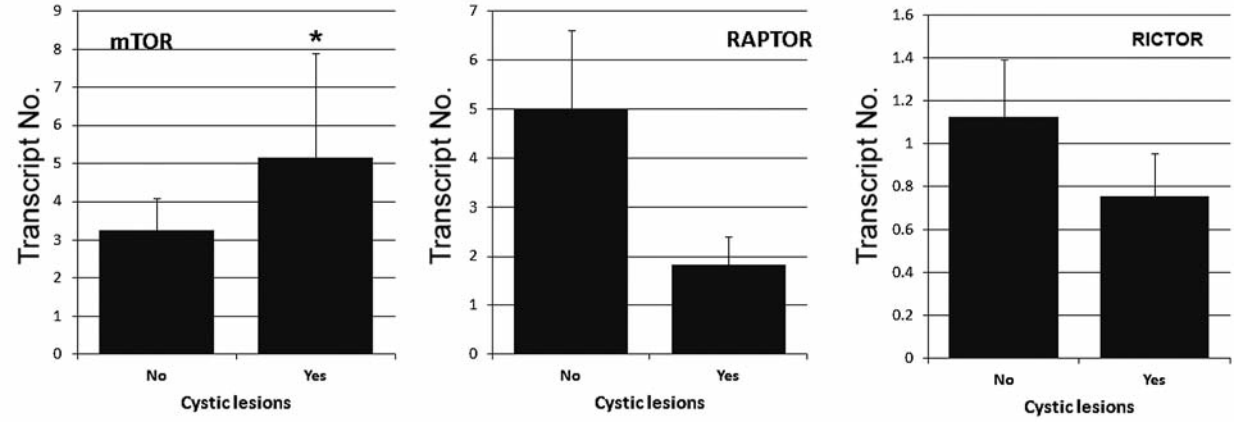

Figure 2. *Levels of Mammalian Target Of Rapamycin (mTOR), Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR (RICTOR) differ with the appearance of cystic lesion in pituitary tumours. mTOR levels were significantly higher in tumours with cystic lesion. RAPTOR and RICTOR levels were marginally lower in tumours with cystic lesion, although those differences were not significant.*

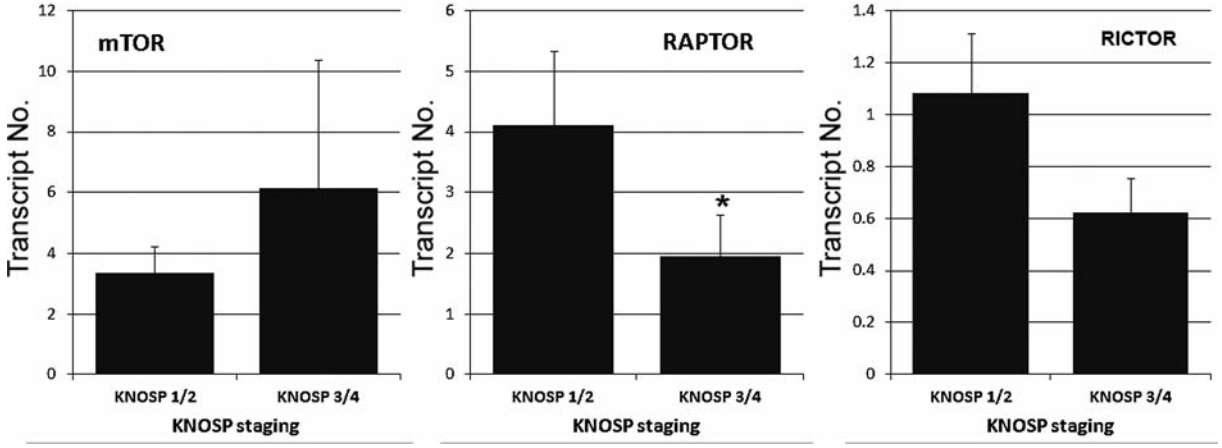

Figure 3. *Levels of Mammalian Target Of Rapamycin (mTOR), Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR (RICTOR) are correlated with the cavernous sinus invasion (Knosp stage). *The level of the RAPTOR transcripts for Knosp stage 3/4 was significantly lower than stage 1-2. A similar trend was seen with RICTOR and an opposite trend was seen with mTOR.*

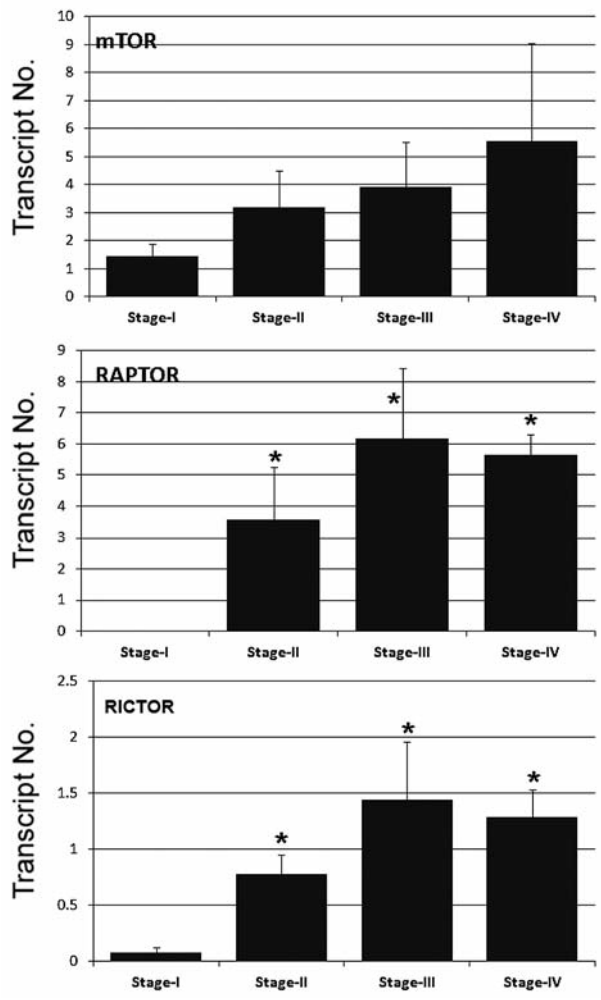

Figure 4. *Levels of Mammalian Target Of Rapamycin (mTOR). Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR (RICTOR) are correlated with the Hardy's tumour stage in pituitary adenomas. *The levels of the respective transcripts in high stage groups were significantly different from these of low tumour stage.*

invasion was tumour growth toward the suprasellar region with invasion in the third ventricle and/or lateral ventricle. The criterion for sphenoid sinus invasion was tumour growth downward to the sphenoid sinus cavity or tumour growth into the clivas. Cavernous sinus invasion was defined as tumour extending lateral to the lateral tangent of the intra- and supracavernous internal carotid artery (ICA) (grade 0-2) or beyond that (grade 3 or 4), with reference to the classification proposed by Knosp et al. (21). Hardy's classification includes four tumour stages, in which tumour size and invasion were both taken into account, these were used in the present study (22). Full details of the clinicopathological information are given in Table I.

Collection of pituitary adenomas. Pituitary adenoma samples were freshly-collected immediately after microsurgical resection at the Department of Neurosurgery of Beijing TianTan Hospital, supported by an ethics approval by the Local Research Ethics Committee with patient's consent. A total of 95 patients entered the current study. The tissues were immediately frozen and stored in liquid nitrogen until use.

Tissue processing and generation of genetic materials for genetic based analyses. Frozen tumour tissues were frozen-sectioned at 10 μm. A small number of sections were used for histological evaluation and the rest were combined and then homogenised in an RNA extraction solution. Total RNA was extracted based on the manufacturer's instructions (Sigama-Aldrich Inc, St Louis, MO, USA) and quantified using a spectrophotometer. Equal amounts of total RNA was used to generate complementary DNA, cDNA, using a reverse transcription kit from Sigma (Sigma-Aldrich Inc, St Louis, MO, USA). The quality of cDNA samples were verified using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a house keeping control.

Quantitative gene transcript analyses. This was based on the Amplifluor™ Technology in quantitative gene transcript analysis. Briefly, pairs of specific primers were designed to amplify a region of mTOR, RAPTOR and RICTOR. To one primer of the primer sets was added a Z sequence which is complementary to the Amplifluor probe (FAM tagged, synthesised by Biosearch Technologies, Inc., Novato, CA, USA). Each reaction comprised of a forward primer, a reserve Z primer at one tenth of the strength, the Amplifluor probe, cDNA from tumours and custom master mix (Life Technologies, Paisley, UK) (Table II). An internal standard was included as control. Quantitative Polymerase Chain Reaction (PCR) analysis was conducted using a SteponePlus instrument (ABI, Paisley, Scotland, UK). GAPDH transcript, was also simultaneously quantified and was used as a house keeping control.

Statistical analysis. The statistical package used was SigmaPlot (Version 11). Student *t*-test and ANOVA were used for normalised data and Mann-Whitney *U*-test and Kruskall–Walis test for non-normalised data.

Results

mTOR, RAPTOR and RICTOR expression and tumour size. There was a stepwise increase in the levels of mTOR and RAPTOR with increasing tumour size (Figure 1), although the differences were not significant. It is interesting to note that there was a similar but significant positive correlation between RICTOR and tumour size, namely $p=0.0012$ and $p=0.0055$ for tumours 1-2 cm and tumours >3 cm compared with tumours <1 cm (Figure 1 and Table III).

Expression of mTOR, RAPTOR and RICTOR was not significantly associated with sex and age (Table III). Significantly higher levels of mTOR were seen in tumours with cystic lesions ($p=0.044$ *vs*. tumours without cysts). Interestingly, RAPTOR and RICTOR levels were marginally lower in tumours with cystic lesions, although these differences were not significant (Figure 2). There was no correlation between mTOR, RAPTOR or RICTOR and intratumour haemorrhage (Table III).

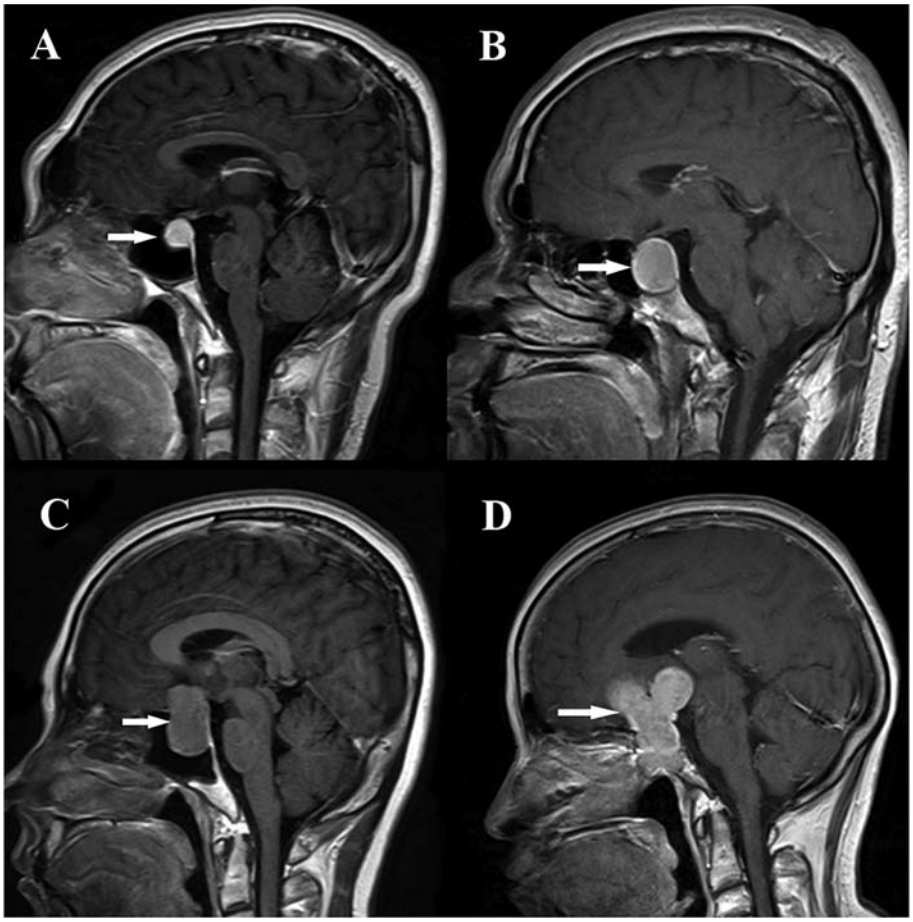

Figure 5. *MRI images of typical cases of Hardy's stage I-IV* (*A-D, respectively), with stepwise increasing levels of Mammalian Target Of Rapamycin (mTOR). Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR* (*RICTOR*). *White arrowhead indicates the tumour.*

mTOR, RAPTOR and RICTOR and invasion. We did not find any correlation between mTOR, RAPTOR or RICTOR with suprasephanous spread and invasion (Table III). There were also no significant difference between tumours with sella floor destruction and without destruction for all three molecules (Table III). Advanced pituitary tumours, namely Knosp stage 3/4, had significantly lower levels of RAPTOR than those of Knosp stage 1/2 (p=0.010). A similar trend was seen for RICTOR although this was not significant (p=0.09). An opposite but insignificant trend was seen for mTOR (p=0.53) (Figure 3).

mTOR, RAPTOR and RICTOR and tumour staging. A modified Hardy's staging showed a clear trend of increased levels of all three molecules with increasing tumour stage (Figure 4). It is interesting to note that stage-I tumours had very little expression of RAPTOR, significantly lower than stage II-IV tumours (p<0.01 stage-I *vs*. other stages) (Figure 4 middle). Likewise, the level was also low in stage-I tumours: Stage-II, III and IV tumours had significantly higher levels of RICTOR transcript than stage-I tumours (p=0.004, 0.014 and 0.006 respectively) (Figure 4 bottom). Transcript levels of mTOR had a similar trend to that of RAPTOR and RICTOR, although this was not statistically significant (p=0.065, stage-I *vs*. other stages). Figure 5 shows some representative MRI images from patients with differing levels of mTOR, RAPTOR, RICTOR and different tumour staging.

mTOR, RAPTOR and RICTOR and endocrine functions. mTOR and RAPTOR levels differed in tumours with different endocrine functions, although no statistical difference was observed. However, GH-, FSH-, TSH-secreting tumours had significantly lower levels of RICTOR compared with non-functional tumours (Figure 6).

mTOR, RAPTOR and RICTOR are intercorrelated. As shown in Table IV and Figure 7, mTOR was found to be

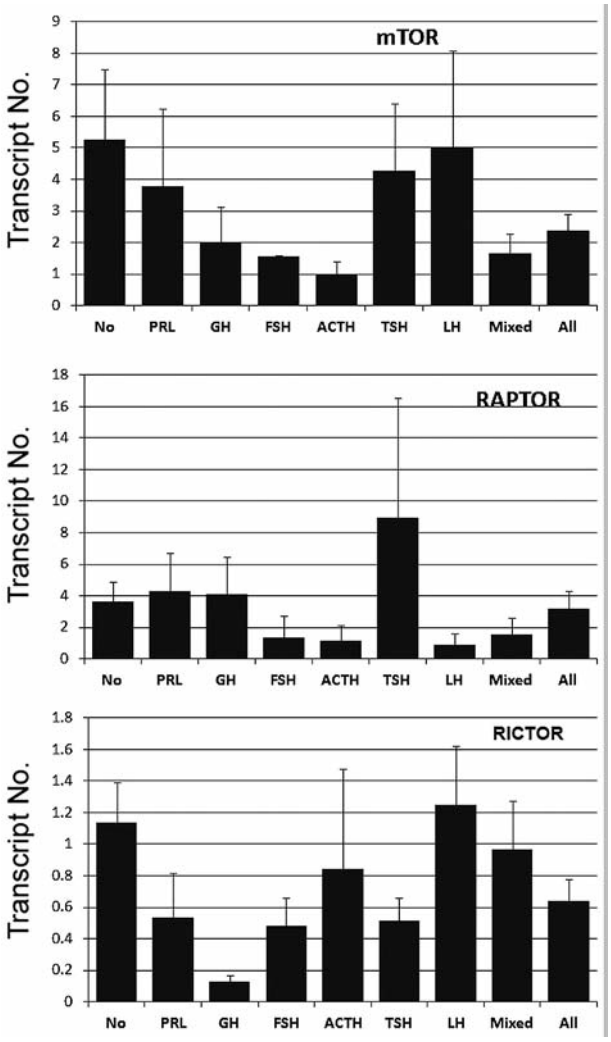

Figure 6. *Expression of Mammalian Target Of Rapamycin (mTOR). Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR (RICTOR) in relation to the endocrine function of pituitary tumours. Growth Hormone (GH)-, Follicle-Stimulating Hormone (FSH)-, Thyroid Stimulating Hormone (TSH)-secreting tumours had significantly low levels of RICTOR compared with non-functional tumours. PRL: Prolactin; ACTH: Adreno-Cortico-Tropic-Hormone; LH: Luteinizing Hormone.*

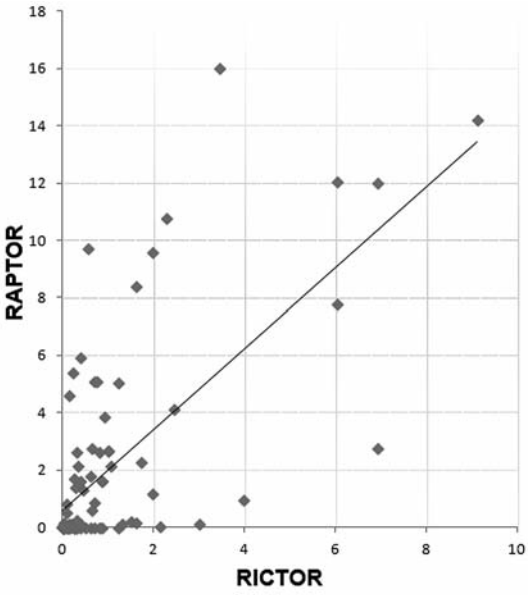

Figure 7. *Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR (RICTOR) were highly significantly correlated.*

Table IV. *Spearman's rank correlations of Mammalian Target Of Rapamycin (mTOR), Regulatory associated protein of mTOR (RAPTOR) and Rapamycin Independent Companion of mTOR (RICTOR).*

	RICTOR	RAPTOR
mTOR	R=0.616 *p*=0.0000002	R=0.371 *p*=0.000234
RICTOR		R=0.659 *p*<0.0000002

significantly correlated with both RAPTOR and RICTOR. It is noteworthy that RAPTOR and RICTOR were found to be highly significantly correlated.

Discussion

The mTOR pathway plays a pivotal role in the regulation of cell growth and death, energy, lipid and protein metabolism and cell migration. Its expression is aberrant in a number of human solid tumour and has been shown to be a viable target in cancer treatment (23-25). Here, we provide fresh evidence that both mTOR and its protein complex facilitators, RAPTOR and RICTOR which form different mTOR complexes, are also aberrantly expressed in human pituitary adenomas.

The most interesting finding of the present study is the link between mTOR, RAPTOR and in particular RICTOR with tumour staging. With increasing tumour stage, there were stepwise increases in the transcript levels of mTOR, RAPTOR and RICTOR. Hardy's classification divides the tumours into four stages by taking into consideration tumour size and invasion. Thus, Hardy staging and the Knosp staging methods are quite different, with the latter being a practical classification for cavernous sinus invasion.

The tumour size is closely related to the clinical symptoms. With an increase in tumour size, patients may first lose visual acuity. With subsequent invasion into the thalamus, the tumour can cause diabetes insipidus. Blocking

the interventricular foramen can lead to hydrocephalus and invasion into the carvernous sinus will cause cranial nerve palsy. Thus, there is a certain relationship between the changes of clinical symptoms in patients with changes in mTOR pathway. The mTOR pathway can promote the proliferation of tumour cells. Strong proliferation of tumour cells is precisely the material basis of pituitary adenoma recurrence and invasive growth. The data presented here, together with the limited reports in the literature, strongly argue that mTOR, RAPTOR and RICTOR have an important predictive and prognostic value in patients with pituitary tumours (25-27).

The finding that RAPTOR and RICTOR expression are both increased together with mTOR is also interesting. RAPTOR and RICTOR form mutually exclusive protein complexes with the mTOR protein. In the protein complexes mTORC1 and mTORC2, there are different components largely as a result of the association between mTOR with either RAPTOR or RICTOR (2-4, 23). It has been established that the two protein complexes have different regulatory roles in the cells, namely mTORC1 is more dominant than mTORC2 in regulating lipid and protein metabolism, cell growth and gene expression. In contrast, mTORC2 is more dominant in regulating cytoskeletal organisation and thus cell migration, as well as activation of mTORC1. The two protein complexes differ in their protein components and in some aspects of cell functions, but also communicate with each other. Thus, the findings that both expression of RAPTOR and RICTOR were highly raised in aggressive pituitary tumours may suggest that both play a key role in pituitary adenoma.

The other intriguing finding is that mTOR expression is significantly correlated with that of both RAPTOR and RICTOR. It is also interesting to observe that RAPTOR and RICTOR are also highly correlated. Presently, the mechanism(s) by which the aberrant expression occurs is not clear. Those three genes are located on different chromosomes. RAPTOR is located on *17q25.3*, RICTOR on chromosome 5, and mTOR on 1p36.22 (28-30). The regulation of gene transcription of all three genes is yet to be fully established. Given that the raised levels of all the molecules are seen at message level, a possibility exists that there may be transcriptional aberration in pituitary adenomas. Further investigation is necessary to establish whether this is the case.

mTOR inhibitor has been indicated in both endocrine-active and -inactive pituitary tumours (24-26). The present study did not show a consistent pattern of the expression of these molecules and the endocrine functions of the tumours. From the above data, we argue that mTOR, RAPTOR and RICTOR have no clear link with the endocrine functions of the tumours, however, a large cohort is required to firmly establish this.

In conclusion, the current study adds further weight the ongoing development and indicates that pituitary adenomas, endocrine-active or -inactive, have aberrant expression of mTOR and its regulator, and appear to be an ideal tumour type for mTOR therapies.

Acknowledgements

The Authors wish to thank Cancer Research Wales and the Albert Hung Foundation for supporting their work.

References

1 Ballou LM and Lin RZ: Rapamycin and mTOR kinase inhibitors. Journal of Chemical Biol *1*: 27-36, 2008.
2 DeBerardinis RJ, Lum JJ, Hatzivassiliou G and Thompson CB: The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab *7*: 11-20, 2008.
3 Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P and Hall MN: Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol cell *10*: 457-468, 2002.
4 Nojima H, Tokunaga C, Eguchi S, Oshiro N, Hidayat S, Yoshino K, Hara K, Tanaka N, Avruch J and Yonezawa K: The mammalian target of rapamycin (mTOR) partner, RAPTOR, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. J Biol Chem *278*: 15461-15464, 2003.
5 Dunlop EA and Tee AR: Mammalian target of rapamycin complex 1: signalling inputs, substrates and feedback mechanisms. Cell Signall *21*: 827-835, 2009.
6 Kaiser CA and Schekman R: Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. Cell *61*: 723-733, 1990.
7 Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A and Hall MN: Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biology *6*: 1122-1128, 2004.
8 Levy S, Avni D, Hariharan N, Perry RP and Meyuhas O: Oligopyrimidine tract at the 5' end of mammalian ribosomal protein mRNAs is required for their translational control. Proc Nati Acad Sci list *88*: 3319-3323, 1991.
9 Meric-Bernstam F and Gonzalez-Angulo AM: Targeting the mTOR signaling network for cancer therapy. J Clin Oncol *27*: 2278-2287, 2009.
10 Lin TA, Kong X, Haystead TA, Pause A, Belsham G, Sonenberg N and Lawrence JC Jr.: PHAS-I as a link between mitogen-activated protein kinase and translation initiation. Science *266*: 653-656, 1994.
11 Svitkin YV, Pause A, Haghighat A, Pyronnet S, Witherell G, Belsham GJ and Sonenberg N: The requirement for eukaryotic initiation factor 4A (elF4A) in translation is in direct proportion to the degree of mRNA 5' secondary structure. RNA *7*: 382-394, 2001.
12 Pickering BM and Willis AE: The implications of structured 5' untranslated regions on translation and disease. Semi Cell Develop Biol *16*: 39-47, 2005.
13 Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML and McCutcheon IE: The prevalence of pituitary adenomas: A systematic review. Cancer *101*: 613-619, 2004.

14 Dai C, Zhang B, Liu X, Ma S, Yang Y, Yao Y, Feng M, Bao X, Li G, Wang J, Guo K, Ma W, Xing B, Lian W, Xiao J, Cai F, Zhang H and Wang R: Inhibition of PI3K/AKT/mTOR pathway enhances temozolomide-induced cytotoxicity in pituitary adenoma cell lines *in vitro* and xenografted pituitary adenoma in female nude mice. Endocrinology *154*: 1247-1259, 2013.
15 Dworakowska D, Wlodek E, Leontiou CA, Igreja S, Cakir M, Teng M, Prodromou N, Goth MI, Grozinsky-Glasberg S, Gueorguiev M, Kola B, Korbonits M and Grossman AB: Activation of RAF/MEK/ERK and PI3K/AKT/mTOR pathways in pituitary adenomas and their effects on downstream effectors. Endocrine-related Cancer *16*: 1329-1338, 2009.
16 Rubinfeld H and Shimon I: PI3K/AKT/mTOR and RAF/MEK/ERK signaling pathways perturbations in non-functioning pituitary adenomas. Endocrine *42*: 285-291, 2012.
17 Sajjad EA, Zielinski G, Maksymowicz M, Hutnik L, Bednarczuk T and Wlodarski P: mTOR is frequently active in GH-secreting pituitary adenomas without influencing their morphopathological features. Endocrin Pathol *24*: 11-19, 2013.
18 Gorshtein A, Rubinfeld H, Kendler E, Theodoropoulou M, Cerovac V, Stalla GK, Cohen ZR, Hadani M, and Shimon I: Mammalian target of rapamycin inhibitors rapamycin and RAD001 (everolimus) induce antiproliferative effects in GH-secreting pituitary tumor cells *in vitro*. Endocrine-related Cancer *16*: 1017-1027, 2009.
19 Sukumari-Ramesh S, Singh N, Dhandapani KM and Vender JR: mTOR inhibition reduces cellular proliferation and sensitizes pituitary adenoma cells to ionizing radiation. Surg Neurol Int *2*: 22, 2011.
20 Wazir U, Newbold RF, Jiang WG, Sharma AK and Mokbel K: Prognostic and therapeutic implications of mTORC1 and RICTOR expression in human breast cancer. Oncol Rep *29*: 1969-74, 2013.
21 Knosp E, Steiner E, Kitz K and Matula C: Pituitary adenomas with invasion of the cavernous sinus space: A magnetic resonance imaging classification compared with surgical findings. Neurosurgery *33*: 610-617; discussion 617-618, 1993.
22 Hardy J: Transsphenoidal surgery of hypersecreting pituitary tumors. *In*: Diagnosis and Treatment of Pituitary Tumors, Kohler PO and Ross GT (eds.). Amsterdam, Exerpta Medica, pp: 179-198, 1973.
23 Laplante M and Sabatini DM: mTOR signaling at a glance. J Cell Sci *122*: 3589-3594, 2009.
24 Lee M, Theodoropoulou M, Graw J, Roncaroli F, Zatelli MC and Pellegata NS: Levels of p27 sensitize to dual PI3K/mTOR inhibition. Mole Cancer Ther *10*: 1450-1459, 2011.
25 Lu C, Willingham MC, Furuya F and Cheng SY: Activation of phosphatidylinositol 3-kinase signaling promotes aberrant pituitary growth in a mouse model of thyroid-stimulating hormone-secreting pituitary tumors. Endocrinology *149*: 3339-3345, 2008.
26 Dowling RJ, Topisirovic I, Fonseca BD, and Sonenberg N: Dissecting the role of mTOR: lessons from mTOR inhibitors. Biochim Biophys Acta *1804*: 433-439, 2010.
27 Jouanneau E, Wierinckx A, Ducray F, Favrel V, Borson-Chazot F, Honnorat J, Trouillas J, and Raverot G: New targeted therapies in pituitary carcinoma resistant to temozolomide. Pituitary *15*: 37-43, 2012.
28 Zeng J, See AP, Aziz K, Thiyagarajan S, Salih T, Gajula RP, Armour M, Phallen J, Terezakis S, Kleinberg L, Redmond K, Hales RK, Salvatori R, Quinones-Hinojosa A, Tran PT and Lim M: Nelfinavir induces radiation sensitization in pituitary adenoma cells. Cancer Biol Ther *12*: 657-663, 2011.
29 Zhang H, Zhang G, Gonzalez FJ, Park SM and Cai D: Hypoxia-inducible factor directs POMC gene to mediate hypothalamic glucose sensing and energy balance regulation. PLoS Biol *9*: e1001112, 2011.
30 Zoncu R, Efeyan A and Sabatini DM: mTOR: From growth signal integration to cancer, diabetes and ageing. Nat Revi Mol Cell Biol *12*: 21-35, 2011.

Received May 26, 2013
Revised June 16, 2013
Accepted June 18, 2013