The PI3K/AKT/mTOR Signaling Pathway Is Overactivated in Primary Aldosteronism

Hengchuan Su¹, Yanyun Gu², Fengying Li², Qidi Wang², Baoxing Huang¹, Xiaolong Jin³, Guang Ning², Fukang Sun¹*

¹ Department of Urology, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China, ² Department of Endocrinology, Clinical Center of Shanghai Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China, ³ Department of Pathology, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China

Abstract

Background: To date, the available non-invasive remedies for primary aldosteronism are not satisfactory in clinical practice. The phosphoinositide 3-kinase (PI3Ks)/protein kinase B (PKB or AKT)/mammalian target of rapamycin (mTOR) signaling pathway is essential for tumorigenesis and metastasis in many types of human tumors, including renal cancer, adrenal carcinoma and pheochromocytoma. The possibility that this pathway is also necessary for the pathogenesis of primary aldosteronism has not yet been explored. To answer this question, we investigated the activity of the PI3K/AKT/mTOR signaling pathway in normal adrenal glands (NAGs), primary aldosteronism (PA) patients and NCI-H295R cells.

Methodology/Principal Findings: Between January 2005 and December 2011, we retrospectively reviewed the records of 45 patients with PA. We compared clinical characteristics (age, gender and biochemical data) and the expression of phospho-AKT (p-AKT), phospho-mTOR (p-mTOR), phospho-S6 (p-S6) and vascular endothelial growth factor (VEGF) by immunohistochemical staining and western blotting, analyzing 30 aldosterone-producing adenomas (APAs), 15 idiopathic hyperaldosteronism (IHA) tissues and 12 NAGs following nephrectomy for renal tumors (control group). Compared with the control group, most of the PA patients presented with polydipsia, polyuria, resistant hypertension, profound hypokalemia, hyperaldosteronemia and decreased plasma renin activity. Compared with normal zona glomerulosa, the levels of p-AKT, p-mTOR, p-S6 and VEGF were significantly upregulated in APA and IHA. No significant differences were found between APA and IHA in the expression of these proteins. Additionally, positive correlations existed between the plasma aldosterone levels and the expression of p-AKT and p-mTOR. In vitro studies showed that mTOR inhibitor rapamycin could inhibit cell proliferation in NCI-H295R cells in a dose- and time-dependent manner. Furthermore, this inhibitor also decreased aldosterone secretion.

Conclusions: Our data suggest that the PI3K/AKT/mTOR signaling pathway, which was overactivated in APA and IHA compared with normal zona glomerulosa, may mediate aldosterone hypersecretion and participate in the development of PA.

Citation: Su H, Gu Y, Li F, Wang Q, Huang B, et al. (2013) The PI3K/AKT/mTOR Signaling Pathway Is Overactivated in Primary Aldosteronism. PLoS ONE 8(4): e62399. doi:10.1371/journal.pone.0062399

Editor: Swati Palit Deb, Virginia Commonwealth University, United States of America

Received December 1, 2012; Accepted March 20, 2013; Published April 23, 2013

Copyright: © 2013 Su et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by Shanghai Municipal Natural Science Foundation (No. 10411960000). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: sunfukang6@126.com

Introduction

Primary aldosteronism (PA) is the most common form of endocrine hypertension, accounting for up to 5% to 10% of all hypertensive patients. It is characterized by the excessive and autonomous secretion of aldosterone by the adrenal gland, and aldosterone-producing adenomas (APAs) and idiopathic hyperaldosteronism (IHA) are the major forms [1,2]. In the last few years, significant advances have been made in the treatment of these two subtypes of PA. Patients with APA can be cured or experience significant amelioration following unilateral adrenalectomy, whereas patients with IHA can benefit from targeted pharmacotherapy with mineralocorticoid receptor antagonists [3]. However, some patients cannot tolerate surgery due to their physical condition, and treatment with medication may cause side effects, including gynecomastia, erectile dysfunction, low libido and irregular menstruation.

The phosphoinositide 3-kinase (PI3Ks)/protein kinase B (PKB or AKT)/mammalian target of rapamycin (mTOR) signaling pathway is a major pathway involved in the regulation of cell proliferation and has therefore become a focus of tumor research in recent years [4,5]. Overactivation of the PI3K/AKT/mTOR pathway, characterized by the production of phospho-AKT (p-AKT), phospho-mTOR (p-mTOR), phospho-S6 (p-S6) and vascular endothelial growth factor (VEGF), occurs in many tumors such as renal cancer, adrenal carcinoma and pheochromocytoma, but it has not been examined in PA [6,7]. Previous studies have provided evidence that the PI3K/AKT pathway stimulates aldosterone secretion in the glomerulosa cells of bovine
adrenal glands through the activity of sphingosine-1-phosphate [8] and that teratocarcinoma-derived growth factor-1 (TDGF-1) associated with the PI3K/AKT pathway is significantly upregulated in human APA and mediates NCI-H295R cell aldosterone hypersecretion [9].

Based on the above evidence, the present study was undertaken to investigate whether the downstream mTOR pathway was overactivated in APA and IHA, in an attempt to understand the functional role of the PI3K/AKT/mTOR pathway with regard to the autonomous secretion of aldosterone and the alterations in cell growth observed in these two subtypes. Additionally, we used an in vitro analysis to evaluate the effects of mTOR inhibitors on cellular proliferation and aldosterone hypersecretion using NCI-H295R cells.

Materials and Methods

Patients and Tissue Samples

Tumor tissues from 45 PAs were collected from patients who underwent adrenalectomy at Ruijin Hospital between January 2005 and December 2011 and were divided into two groups: 30 APAs and 15 IHA tissues. The 30 PA patients included 12 males and 18 females, ranging from 42 to 55 years of age. The 15 IHA patients included 6 males and 9 females, ranging from 38 to 57 years of age. The PA pathology specimens used in this study were removed from patients studied in our hypertension unit who had been homogeneously selected following a rigorous diagnostic differentiation diagnosis of APA and IHA and had to receive the surgery for the treatment and diagnosis. The influences of prior treatment on the two groups was the same. Ipsilateral normal adrenal glands (NAGs) from 12 cases undergoing nephrectomy for renal tumors served as the control group. All tissue specimens were collected with written informed consent, and approval was obtained from the institutional ethics review board of Ruijin Hospital.

Cell Culture

NCI-H295R cells were purchased from American Type Culture collection (ATCC, Manassas, VA, USA), cultured in DMEM/F12 supplemented with 2.5% nu-serum replacements, 1% ITS+ Premix, 1% l-glutamine and 1% penicillin-streptomycin (Gibco Invitrogen, Carlsbad, CA, USA) and maintained at 37°C in a humidified 5% CO2 incubator. We tested the effects of rapamycin (Cell Signaling Technologies, Danvers, MA, USA) on cell growth with the concentrations of 25 nM, 50 nM, 100 nM after 24 h, 48 h and 72 h treatment. Culture media were collected and assayed for aldosterone by radio-immunoassay after 48 h of incubation as described previously [10]. Aldosterone measurements were normalized using the total protein concentrations of cell lysates. DMSO was used as a vehicle. All experiments were performed in triplicate and repeated 3 times.

Table 1. The demographics and clinical outcomes of the patients.

| Variables                        | APA     | IHA     | NAG     | P value  |
|----------------------------------|---------|---------|---------|----------|
|                                  | APA VS NAG | IHA VS NAG | IHA VS APA |         |
| Age (years)                      | 51.2 ± 8.5 | 52.6 ± 6.5 | 49.2 ± 4.3 | 0.227    | 0.219    | 0.098   |
| Sex, M/F                         | 12/18   | 6/9     | 5/7     | 1        | 1        | 1       |
| Blood pressure                   |         |         |         |          |          |
| Systolic blood pressure (mmHg)   | 167.4 ± 6.1 | 158.2 ± 4.9 | 148.4 ± 6.7 | 0.359    | 0.893    | 0.403   |
| Diastolic blood pressure (mmHg)  | 107.1 ± 5.9 | 105.6 ± 5.1 | 97.8 ± 5.4 | 0.536    | 0.948    | 0.583   |
| Water balance                    |         |         |         |          |          |
| Oral water intake (mL)           | 2193 ± 92 | 1927 ± 110 | 1379 ± 87 | 0.037    | 0.013    | 0.732   |
| Urine volume (mL)                | 2765 ± 114 | 2583 ± 75  | 1728 ± 96  | 0.038    | 0.008    | 0.211   |
| Serum physiological parameters   |         |         |         |          |          |
| Serum potassium (mmol/L)         | 2.47 ± 0.21 | 2.81 ± 0.32 | 4.56 ± 0.55 | 0.003    | 0.007    | 0.004   |
| Serum aldosterone (pmol/L)       | 872 ± 190 | 785 ± 189 | 287 ± 146 | 0.021    | 0.015    | 0.037   |
| Basis plasma renin activity (ng·mL⁻¹·h⁻¹) | 0.14 ± 0.18 | 0.21 ± 0.35 | 1.49 ± 0.25 | 0.041    | 0.038    | 0.789   |
| Serum sodium (mmol/L)            | 146.8 ± 1.7 | 142.9 ± 2.7  | 137.3 ± 3.2 | 0.578    | 0.427    | 0.874   |
| Serum chloride (mmol/L)          | 101.8 ± 3.1 | 99.5 ± 2.4   | 98.8 ± 3.5  | 0.753    | 0.668    | 0.947   |
| Urinary physiological parameters |         |         |         |          |          |
| 24 h urinary potassium (mmol/L)  | 115.3 ± 29.8 | 93.3 ± 33.6 | 64.4 ± 22.3 | 0.001    | 0.043    | 0.038   |
| 24 h urinary aldosterone (ug)    | 37.1 ± 5.6 | 32.6 ± 7.2  | 15.4 ± 4.9  | 0.029    | 0.038    | 0.041   |
| 24 h urinary sodium (mmol/L)     | 202.6 ± 35.3 | 188.1 ± 29.8 | 215.4 ± 47.7 | 0.493    | 0.387    | 0.582   |
| 24 h urinary chloride (mmol/L)   | 189.6 ± 56.9 | 217.7 ± 43.5 | 202.5 ± 67.5 | 0.294    | 0.198    | 0.781   |

APA aldosterone-producing adenomas; IHA idiopathic hyperaldosteronism; NAG normal adrenal gland.
doi:10.1371/journal.pone.0062399.t001
Immunohistochemistry

Specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were incubated with polyclonal rabbit antihuman p-AKT, p-mTOR, p-S6 (Cell Signaling Technologies, Danvers, MA, USA, 1:150) and VEGF antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:400) and were developed using diaminobenzidine (DAB) substrate. The slides were examined independently by two investigators who were blinded to both the clinical and pathological data. Protein expression was quantified based on the extent of staining (percent of positive tumor cells graded on a scale of 0 to 4: 0, none; 1, 1%–25%; 2, 26%–50%; 3, 51%–75%; 4, >75%) and staining intensity (graded on a scale of 0 to 3: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining). For further analysis, we used the product of the grades of the extent and intensity of staining to define the cutoff value to classify protein expression as high (grades 4–12: ++; 7–9, +++; 10–12, ++++) or low (grade 0–3: –).

Western Blotting

Frozen adrenal gland samples were centrifuged, and the soluble protein concentrations were determined before loading on a gel. Antibodies against the following antigens were used: p-AKT, p-S6, β-actin antibody (Cell Signaling Technologies, Danvers, MA, USA, 1:2000) and VEGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:1000). Protein bands were visualized using an enhanced chemiluminescence assay kit (SuperSignal Pierce Biotechnology, USA). The protein bands were identified by comparison with known molecular weight markers.

Statistical Analysis

Data analyses were performed using SPSS statistical package 15.0 (SPSS, Inc, Chicago, IL, USA). The significance of differences in the measurement data were evaluated by ANOVA, while enumerable data were analyzed using the chi-square test and independent samples T test. Ranked data were analyzed by the

Table 2. Summary of the immunohistochemical staining results for p-AKT, p-mTOR, p-S6 and VEGF in APA, IHA and NAG tissues.

| Proteins | Group | APA | IHA | NAG | \( p \) value | APA VS NAG | IHA VS NAG | IHA VS APA |
|----------|-------|-----|-----|-----|-------------|------------|------------|------------|
| p-AKT    | High  | 22  | 11  | 3   | 0.011       | 0.021      | 1          |
|          | Low   | 8   | 4   | 9   |             |            |            |
| p-mTOR   | High  | 25  | 12  | 4   | 0.005       | 0.022      | 1          |
|          | Low   | 5   | 3   | 8   |             |            |            |
| p-S6     | High  | 26  | 11  | 4   | 0.002       | 0.057      | 0.491      |
|          | Low   | 4   | 4   | 8   |             |            |            |
| VEGF     | High  | 21  | 10  | 2   | 0.002       | 0.019      | 1          |
|          | Low   | 9   | 5   | 10  |             |            |            |

APA aldosterone-producing adenomas; IHA idiopathic hyperaldosteronism; NAG normal adrenal gland.

doi:10.1371/journal.pone.0062399.g001

doi:10.1371/journal.pone.0062399.t002

Figure 1. Expression of the PI3K/AKT/mTOR signaling pathway by immunohistochemistry in NAGs, APA and IHA patients. NAGs served as a reference. In NAGs, faint staining of these proteins is visible in the cytoplasm of normal zona glomerulosa cells. However, in IHA and APA patients, the staining of these four proteins is stronger. In general, the immunohistochemical staining of the tissue sections indicated that p-AKT, p-mTOR, p-S6 and VEGF were highly expressed in APA and IHA sections compared with NAGs. (Magnification × 400).

doi:10.1371/journal.pone.0062399.g001
rank sum test and the Spearman correlation test. The results were considered significant if the P-value was less than 0.05. The quantitative data were expressed as the mean ± SD.

Results

Summary of Clinical Characteristics

Table 1 lists the clinical features and the results of biochemical examination of PA patients. Most of the patients presented with polydipsia, polyuria, resistant hypertension, profound hypokalemia, hyperaldosteronemia and decreased plasma renin activity. Significant differences were observed between the control group and PA groups in oral water intake, urine volume, plasma renin activity, serum potassium and serum aldosterone. The urinary potassium and aldosterone between the control group and PA groups were also significantly different. The aldosterone level of APA patients in serum and urine were higher than those of IHA patients, while potassium level (serum and urine) of APA patients were lower than that of IHA patients (P < 0.05). No significant differences were found in other physiological parameters between APA and IHA patients (Table 1).

mTOR Signaling Pathway was Overactivated in Adrenal Tissues of Primary Aldosterone Patients

At the microscopic level, typical cell patterns were observed in the diseased adrenal tissues of IHA and APA patients. These cells were cuboidal or low columnar in shape and were arranged in spherical masses. The volumes of the cells and nuclei were smaller than the zona fasciculata, with fewer lipid droplets (Figure 1). Immunohistochemical staining of tissue sections demonstrated that p-AKT, p-mTOR, p-S6 and VEGF were predominantly expressed in the cytoplasm of APA and IHA tissues. In contrast, only faint protein labeling was visible in the cytoplasm of normal zona glomerulosa cells (Figure 1). Table 2 showed these four proteins were significantly upregulated in APA and IHA tissues compared with normal zona glomerulosa, while no differences were found between APA and IHA tissues.

As shown in table 3, p-AKT and p-mTOR expression was correlated with the plasma aldosterone level (P < 0.05) but not with any other variable. A Spearman correlation analysis indicated that positive correlations existed between the plasma aldosterone level and p-AKT and p-mTOR expression (r_s = 0.356, P < 0.01; r_s = 0.295, P < 0.05).

The results of western blotting were consistent with the immunohistochemical staining. Compared with NAGs, p-AKT, p-S6 and VEGF were highly expressed in APA and IHA tissues (Figure 2). A photodensitometric analysis of the APA and IHA samples revealed elevations of the p-AKT/β-actin, p-S6/β-actin and VEGF/β-actin ratios compared with NAGs (Figure 2). The ratios of p-AKT/β-actin, p-S6/β-actin and VEGF/β-actin significantly differed between NAGs and APA (P < 0.05) and NAGs and IHA (P < 0.05), while no significant differences were observed in these ratios between IHA and APA samples (P = 0.751, 0.302, 0.347), respectively.

Rapamycin Inhibited NCI-H295R Cell Proliferation and Aldosterone Secretion

Since mTOR pathway is activated in PA, we studied the effect of the mTOR inhibitor rapamycin on the proliferation of NCI-H295R cells. Our results showed that rapamycin significantly suppressed cell growth in a dose- and time-dependent manner (Figure 3). A statistically significant reduction in cell proliferation

| Variables | Number | p-AKT | p-mTOR |
|-----------|--------|-------|--------|
| Gender    |        |       |        |
| Male      | 23     | 8     | 9      |
| Female    | 34     | 13    | 17     |
| Age (Years) |      |       |        |
| <50       | 28     | 11    | 9      |
| ≥50       | 29     | 10    | 12     |
| Blood pressure |      |       |        |
| Normal    | 10     | 6     | 3      |
| High      | 47     | 15    | 19     |
| Plasma aldosterone |        |       |        |
| Normal    | 18     | 10    | 7      |
| High      | 39     | 11    | 12     |
| Serum K   |        |       |        |
| Normal    | 17     | 9     | 7      |
| Low       | 40     | 12    | 21     |
| PRA       |        |       |        |
| Normal    | 27     | 9     | 11     |
| Low       | 30     | 12    | 13     |

*indicates that the expression of p-AKT and p-mTOR was correlated with the plasma aldosterone level (P = 0.008, P = 0.027).

doi:10.1371/journal.pone.0062399.t003
with rapamycin treatment was evident at 50 nM and 100 nM (P<0.05). Additionally, aldosterone levels in the supernatant of NCI-H295R cells grown as monolayers were inhibited by rapamycin (48 h of incubation) in a dose-dependent manner. Aldosterone secretion was inhibited by 47.6% in the rapamycin (50 nM)-treated cells (P<0.05) and by 54.6% in rapamycin (100 nM)-treated cells (P<0.01) (Figure 3).

Discussion

AKT, also known as protein kinase B (PKB), is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes such as apoptosis, cell proliferation and cell migration. The predominant roles of mTOR and S6 in mammals are to regulate the function of ribosomes and promote the translation of cell cycle-related proteins [11]. Activation of the mTOR pathway occurs via a multistep process that includes upstream phosphoinositide-3 kinase (PI3K) and AKT activation, eliciting the activation of mTORC1. mTORC1 can also be directly phosphorylated as part of the mTORC1 complex by AKT, forming phospho-mTOR (p-mTOR), which is often studied as a marker of mTOR activation [11]. Activation of the mTORC1 complex leads to the activation of the protein kinase p70 ribosomal protein S6 kinase 1 (S6), which can promote the synthesis of several oncogenic proteins, including c-Myc, hypoxia-inducible factor-1 (HIF-1), VEGF and cyclin D [12,13].

Compared with normal zona glomerulosa, p-AKT expression was significantly upregulated in APA and IHA tissues, which is consistent with a previous study demonstrating that the PI3K/AKT pathway is related to aldosterone secretion in glomerulosa cells [9]. Our results also showed significant differences between NAG and PA tissues in the expression of p-mTOR, p-S6 and VEGF as downstream signaling molecules of p-AKT. Positive correlations existed between the level of plasma aldosterone and the expression of p-AKT and p-mTOR, suggesting that the PI3K/AKT pathway may participate in zona glomerulosa hyperplasia and the hypersecretion of aldosterone via the downstream mTOR pathway.

In vitro, the human NCI-H295R cell line is a commonly utilized model for autonomous aldosterone secretion, although it was derived from an adrenocortical carcinoma and therefore may inherently not reflect the molecular and functional changes of true aldosterone-producing adenomas [14]. Nevertheless, in contrast to other adrenocortical cell lines, such as SW13 cells, these cells are known to be capable of aldosterone production. Some groups have used these cells as a model of PA and aldosterone production [15]. Doghman et al found that p-mTOR was highly expressed in NCI-H295R cells and that mTOR inhibitors could significantly inhibit cell proliferation in vitro and xenograft growth, which was consistent with our results [16]. We also found that rapamycin could decrease aldosterone secretion in NCI-H295R cells, indicating that rapamycin may also have a potential inhibitory effect on PA.

In a recent study evaluating the effects of sorafenib (a VEGFR tyrosine kinase inhibitor) and everolimus (an mTOR inhibitor) in primary adrenocortical cell cultures taken from APA, the authors showed that VEGF and the VEGFR were highly expressed in APA compared with NAGs and that these drugs significantly
inhibited cell growth [17]. Bernini et al also observed higher VEGF expression in APA tissues than NAGs, which was in agreement with our results. Williams et al found that TDGF-1, which belongs to the epidermal growth factor-CFC family of proteins, was significantly upregulated in APA compared with NAGs and played a functional role in aldosterone secretion and protection from apoptosis in NCI-H295R cells via PI3K/Akt signaling [9]. These results all suggest that the PI3K/AKT/mTOR pathway plays an important role in autonomous aldosterone secretion and alterations in cell growth in PA.

The PI3K/AKT/mTOR pathway may participate in zona glomerulosa hyperplasia or aldosterone secretion in the following ways. SREBP-1, a downstream signaling molecule of the PI3K/AKT/mTOR pathway, may participate in the synthesis of lipoidal substances such as cholesterol [18]. The overactivation of this pathway could activate SREBP-1 and generate more cholesterol for the synthesis of aldosterone. At the same time, more calcium ion channels could be activated to trigger aldosterone secretion [19]. Moreover, this pathway could stimulate the synthesis of several oncogenic proteins, such as c-Myc, VEGF and IGF-II, initiating cellular hyperplasia in PA [12–13]. However, further studies are required to test these hypotheses.

No significant differences in the expression of p-AKT, p-mTOR, p-S6 and VEGF were found between IHA and APA tissues, suggesting that the PI3K/AKT/mTOR pathway may play an important role in the development of PA but might not be a marker for differentiating these two subtypes of PA. Changes in the activation of the PI3K/AKT/mTOR pathway may constitute one of several important changes in the pathogenesis of PA, in which the cumulative effects of a number of genetic alterations are required for the initiation of PA formation and the subsequent autonomous hypersecretion of aldosterone. The present study provides evidence for a potential role of this pathway in the pathogenesis of PA and highlights the lack of a significant difference between APA and IHA, thus providing the basis for future studies addressing the role of this pathway in the pathophysiology of PA.

Conclusions

To the best of our knowledge, this is the first detailed study reporting the overactivation of the PI3K/AKT/mTOR pathway in PA. Our data indicate that this pathway may participate in the pathogenesis of PA and may be a potential target for the treatment of this disease. We also found that mTOR inhibitor, rapamycin, could inhibit the proliferation of NCI-H295R cells and reduce aldosterone secretion. Inhibiting the synthesis of aldosterone and zona glomerulosa hyperplasia or neoplasia by blocking this pathway may be a new treatment option for IHA and patients who cannot tolerate the operation.

Author Contributions

Conceived and designed the experiments: FKS YYG QDW HCS. Performed the experiments: HCS. Analyzed the data: HCS BXH. Contributed reagents/materials/analysis tools: GN XLJ FYL. Wrote the paper: HCS YYG.
References

1. Mulatero P, Dluhy RG, Giacchetti G, Boscaro M, Veglio F, et al. (2005) Diagnosis of primary aldosteronism: from screening to subtype differentiation. Trends Endocrinol Metab 16: 114–119.

2. Mulatero P, Stowasser M, Loz KC, Fardella CE, Gordon RD, et al. (2004) Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. J Clin Endocrinol Metab 89: 1045–1050.

3. Stowasser M, Gordon RD, Gunatillaka TG, Cowley DC, Ward G, et al. (2003) High rate of detection of primary aldosteronism, including surgically treatable forms, after ‘non-selective’ screening of hypertensive patients. J Hypertens 21: 2149–2157.

4. Guerin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. Cancer Cell 12: 9–22.

5. Konings IR, Verweij J, Wiemer EA, Sleijfer S (2009) The applicability of mTOR inhibition in solid tumors. Curr Cancer Drug Targets 9: 439–450.

6. Hanna SC, Heathcote SA, Kim WY (2008) mTOR pathway in renal cell carcinoma. Expert Rev Anticancer Ther 8: 283–292.

7. De Martino MC, van Koetsveld PM, Pivonello R, Holland IJ (2010) Role of the mTOR pathway in normal and tumoral adrenal cells. Neuroendocrinology 92 (Suppl 1): 28–34.

8. Brizuela L, Ra bano M, Gangoiti P, Narbona N, Macarulla JM, et al. (2007) Sphingosine-1-phosphate stimulates aldosterone secretion through a mechanism involving the PI3K/PKB and MEK/ERK 1/2 pathways. J Lipid Res 48: 2264–2274.

9. Williams TA, Monticone S, Morello F, Liew CC, Mengozzi G, et al. (2010) Teratocarcinoma-derived growth factor-1 is upregulated in aldosterone-producing adenomas and increases aldosterone secretion and inhibits apoptosis in vitro. Hypertension 55: 1468–1473.

10. Mulatero P, Bertello C, Rosato D, Mengozzi G, Milan A, et al. (2008) Roles of clinical criteria, computed tomography scan, and adrenal vein sampling in differential diagnosis of primary aldosteronism subtypes. J Clin Endocrinol Metab 93: 1366–1371.

11. Liu P, Cheng H, Roberts TM, Zhao J (2009) Target-ing the phosphoinositide 3-kinase pathway in cancer. Nat Rev 8: 627–644.

12. Kimura R, Okouchi M, Fujikawa H, Ichi-yamada A, Ryuge F, et al. (2009) Glucagon-like peptide-1 (GLP-1) protects against methylglyoxal-induced PC12 cell apoptosis through the PI3K/Akt/mTOR/GCLc/redox signaling pathway. Neuroscience 162: 1212–1219.

13. Takekoshi K, Isobe K, Yashiro T, Hara H, Ishii K, et al. (2004) Expression of vascular endothelial growth factor (VEGF) and its cognate receptors in human pheochromocytomas. Life Sci 74: 863–871.

14. Rainey WE, Saiuer K, Schimmer BP (2004) Adrenocortical cell lines. Mol Cell Endocrinol 228: 25–38.

15. Lichtenauer UD, Shapiro I, Oswald A, Meurer S, Kalle A, et al. (2012) Characterization of NCI-H295R Cells as an In Vitro Model of Hyperaldosteronism. Horm Metab Res. Epub ahead of print.

16. Doghman M, El Wakil A, Cardinaud B, Thomas E, Wang J, et al. (2010) Regulation of insulin-like Growth Factor-Mammalian Target of Rapamycin Signaling by MicroRNA in Childhood Adrenocortical Tumors. Cancer Res 70: 4666–4675.

17. Marinello B, Rosato A, Zuccolotto G, Rubin B, Cicala MV, et al. (2012) Combination of sorafenib and everolimus impacts therapeutically on adrenocortical tumor models. Endocr Relat Cancer 19: 527–539.

18. Baklan I, Laplante M (2012) Connecting mTORC1 signaling to SREBP-1 activation. Curr Opin Lipidol 23: 226–234.

19. Régimbald-Dumas Y, Frégeau MO, Guillemette G (2011) Mammalian target of rapamycin (mTOR) phosphorylates inositol 1,4,5-triphosphate receptor type 2 and increases its Ca(2+) release activity. Cell Signal 23: 71–79.