OPEN ACCESS

GAVIN PUBLISHERS

Journal of Oncology Research and Therapy

Tsai TS, et al. J Oncol Res Ther: JONT-142.

DOI: 10.29011/2574-710X. 000042

Research Article

PTEN Suppression on RHEB Expression through Inhibition of mTORC1/S6K1-Dependent Protein Translation in Prostate Cancer Cells

Tz-Shiu Tsai[1], Cheng-Keng Chuang[2,3], Da-Chang Chu[1,4,5*]

[1]Graduate Institute of Biomedical Sciences, Chang Gung University, Tao-Yuan, Taiwan

[2]Division of Urology, Department of Surgery, Chang Gung Memorial Hospital, Lin-Kou, Taiwan

[3]Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, Tao-Yuan, Taiwan

[4]Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Tao-Yuan, Taiwan

[5]Department of Pediatrics, Chang Gung Memorial Hospital, Lin-Kou, Taiwan

[*]**Corresponding author:** Da-Chang Chu, Department of Medical Biotechnology and Laboratory Science, Chang Gung University, 259 Wen-Hwa1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan. Tel: +88632118800 Ext-5086; Fax: +88632118512; Email: dcchu@mail.cgu.edu.tw

Citation: Tsai TS, Chuang CK, Chu DC (2018) PTEN Suppression on RHEB Expression through Inhibition of mTORC1/S6K1-Dependent Protein Translation in Prostate Cancer Cells. J Oncol Res Ther: JONT-142. DOI: 10.29011/2574-710X. 000042

Received Date: 02 February, 2018; **Accepted Date:** 23 February, 2018; **Published Date:** 05 March, 2018

Abstract

Purpose: PTEN loss-of-expression and increase of the RHEB protein are frequent oncogenic events in prostate cancer cells. The aim of this study was to elucidate if loss of PTEN leads to the amplification of the RHEB protein in prostate cancer cells.

Materials and Methods: The expression levels of PTEN and RHEB in 2 cell lines (PC-3, and DU 145) 5 pairs of normal prostatic epithelia and prostate tumor samples by real-time quantitative PCR and western blot hybridization.

Results: The RHEB protein level was high in the PC-3 cell line (PTEN-null) and low in the DU 145-cell line (PTEN-expressed). The protein level, but not the mRNA level, of RHEB was up-regulated by PTEN knockdown in DU 145 cells. The increased protein level of RHEB was observed in 5 tumor sample accompanied by decreased protein level of PTEN in 4 paired normal tissues. Inhibitors of PI3K or mTORC1 interrupting the PI3K/AKT1/mTORC1/S6K1 kinase cascade lowered the RHEB protein level in PC-3 cells. The upregulation of the RHEB protein in PTEN-knockdown DU 145 cells was neutralized by inhibition of the PI3K/AKT1/mTORC1/S6K1 kinase cascade or the subsequent protein translation.

Conclusions: These data suggested that the antagonism of PTEN to the PI3K/AKT1 axis leads to the inhibition of the mTORC1/S6K1-mediated protein translation, resulting in the decrease in the RHEB protein level in prostate cancer cells. Our finding provided a new explanation for the aberrant amplification of the RHEB protein in prostate cancer cells.

Keywords: mTORC1 Complex Human; Prostate Cancer; PTEN Protein Human; Protein Translation RHEB Protein Human

Background

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressive lipid phosphatase. It is frequently hypo expressed or dysfunctional during prostate carcinogenesis due to genetic deletion, mutations, or epigenetic regulatory mechanisms [1-3]. PTEN functions as an antagonist of PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase), converting PIP_3 (phosphatidylinositol-3,4,5-triphosphate) to PIP_2 (phosphatidylinositol-4,5-bisphosphate) and consequently interrupting the PDK1 (phosphoinositide-dependent kinase-1)-mediated activation of AKT1 [4,5]. The currently known approaches by which PTEN regulates the expression of other genes include the function of FOXO (forkhead box O class) transcription

factors [6] and the inhibition of protein synthesis [7]. Although the role of PTEN in the kinase cascade has almost been fully explored, its position in the regulation of the downstream target expression is barely understood.

RHEB (Ras Homolog Enriched in Brain) is an oncogenic G-protein which increases aberrantly in prostate oral, lung and skin cancer cells [8-12]. It plays as a crucial activator of the mTORC1 (mammalian target of rapamycin complex 1) protein complex [13,14]. The GAP (GTPase activating protein) activity of the TSC (tuberous sclerosis complex) protein complex is necessary for facilitating the conversion of RHEB from the active status to the inactive one [15, 16]. RHEB is indirectly activated by AKT1 based on the AKT1-dependent inactivation of TSC [17]. As a result, RHEB connects the PI3K/AKT1 pathway and the mTORC1 signaling. The overexpression or uncontrolled activation of RHEB results in the hyperactivation of the mTORC1 signaling, leading to the abnormal facilitation of protein translation, cell growth and proliferation [8,9,15,18,19].

The causes of the RHEB overexpression in prostate cancer cells are not completely understood. It is suggested that the RHEB expression can be abnormally upregulated by genomic alterations and/or post-transcriptional mechanisms in prostate cancer cells [9]. It is likely that PTEN has a negative effect on the expression of RHEB by the inhibition of protein synthesis. The aim of this study was to elucidate the relationship between the loss of PTEN and the amplification of RHEB in prostate cancer cells.

Materials and Methods

Prostate Cancer Specimens

Five pairs of normal prostatic epithelia and prostate tumors were acquired from the tissue bank of Chang Gung Memorial Hospital, Lin-Kou, Taiwan. The protein levels in tumors were compared with that in normal epithelia to determine the change in expression during carcinogenesis.

Cell Lines and siRNA Transfection

Prostate cancer cell lines, PC-3 (ATCC number: CRL-1435) and DU 145 (ATCC number: HTB-81), were cultivated in RPMI 1640 (Invitrogen, Grand Island, NY, USA) supplemented with 10% FBS (Biological Industries, Kibbutz Beit-Haemek, Israel) and 1% mixture of penicillin, streptomycin and amphotericin B (Biological Industries). Following the user's guide of ToolSmartFect reagent (Biotools, New Taipei City, Taiwan), 4×10^5 DU 145 cells seeded on 6-well culture plate were transfected with Dharmacon ON-TARGET plus siRNA pool (Thermo Scientific, Pittsburgh, PA, USA) for 48 hours.

Inhibitors

LY294002 (PI3K inhibitor), rapamycin (mTORC1 inhibitor) and cycloheximide (protein translation inhibitor) were acquired from Sigma-Aldrich (St. Louis, MO, USA) and were dissolved in Dimethyl Sulfoxide (DMSO).

RNA Extraction and Real-Time Quantitative PCR

Total RNA was extracted by Direct-zol RNA Miniprep Kit (Zymo Research, Irvine, CA, USA) and was subjected to cDNA synthesis by ToolScript MMLV RT Kit (Biotools). Real-time PCR was performed with LightCycler 480 SYBR Green I Master (Roche Applied Science, Mannheim, Germany) reagent in LightCycler 480 Instrument (Roche Applied Science) following the manual. Primers specific to RHEB mRNA were customized by Protech Technology (Taipei, Taiwan). Forward primer: 5'-CAATTTGTGGACTCCTAC-3'; reverse primer: 5'-ATATCTATGGAGTATGTC-3'.

Protein Extraction and Western Blot

Homogenized tissues or harvested cells were incubated with RIPA Lysis Buffer (Merck Millipore, Billerica, MA, USA) to prepare protein samples. Electrophoresis was conducted with SDS-containing 12% polyacrylamide gel. Antibodies specific to PTEN (EnoGene, New York, NY, USA), p-AKT1 on T308 (Cell Signaling Technology, Danvers, MA, USA), p-AKT1 on S473 (Cell Signaling Technology), AKT1 (Origene, Rockville, MD, USA), p-S6K1 on T389 (Merck Millipore), S6K1 (Merck Millipore), RHEB (Bioss, Woburn, MA, USA), RICTOR (Merck Millipore) and β-actin (EnoGene) were applied during primary antibody hybridization. Protein levels detected by western blot were quantified by Image J software.

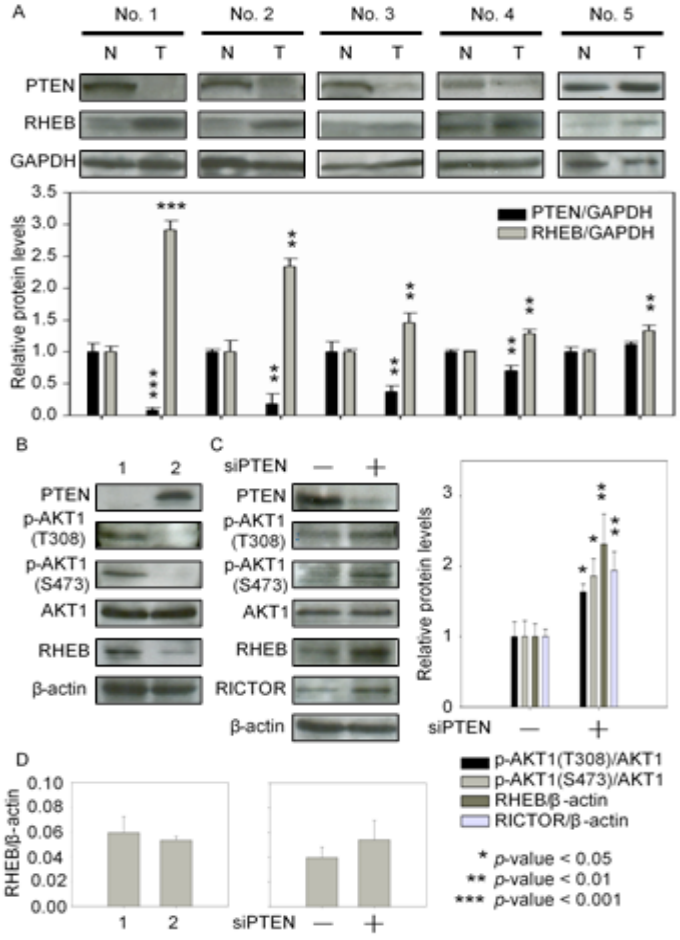
Statistical Analysis

The differences in expression levels of mRNA and protein between samples were analyzed with student's t-test. The significance of differential expression levels was denoted when p-value < 0.05.

Results

Protein levels of PTEN and RHEB were analyzed in 5 pairs of normal prostatic epithelia and prostatic tumors. Loss of PTEN and RHEB overexpression occurred in tumor no. 1 to no. 4 when compared with corresponding normal tissues. However, tumor no. 5 exhibited RHEB amplification without PTEN loss (Figure 1A). To validate the linkage between loss of PTEN and RHEB overexpression, the protein levels of PTEN and RHEB in PC-3 and DU 145 prostate cancer cell lines were analyzed. The PTEN protein was null in PC-3 cells and functionally expressed in DU 145 cells, which was confirmed by the AKT1 phosphorylation levels. In comparison between two cell lines, the RHEB protein displayed a higher level in PC-3 cells and a lower level in DU 145 cells (Figure 1B). PTEN knockdown in DU 145 cells increased the phosphorylated AKT1 and RHEB protein (Figure 1C). The

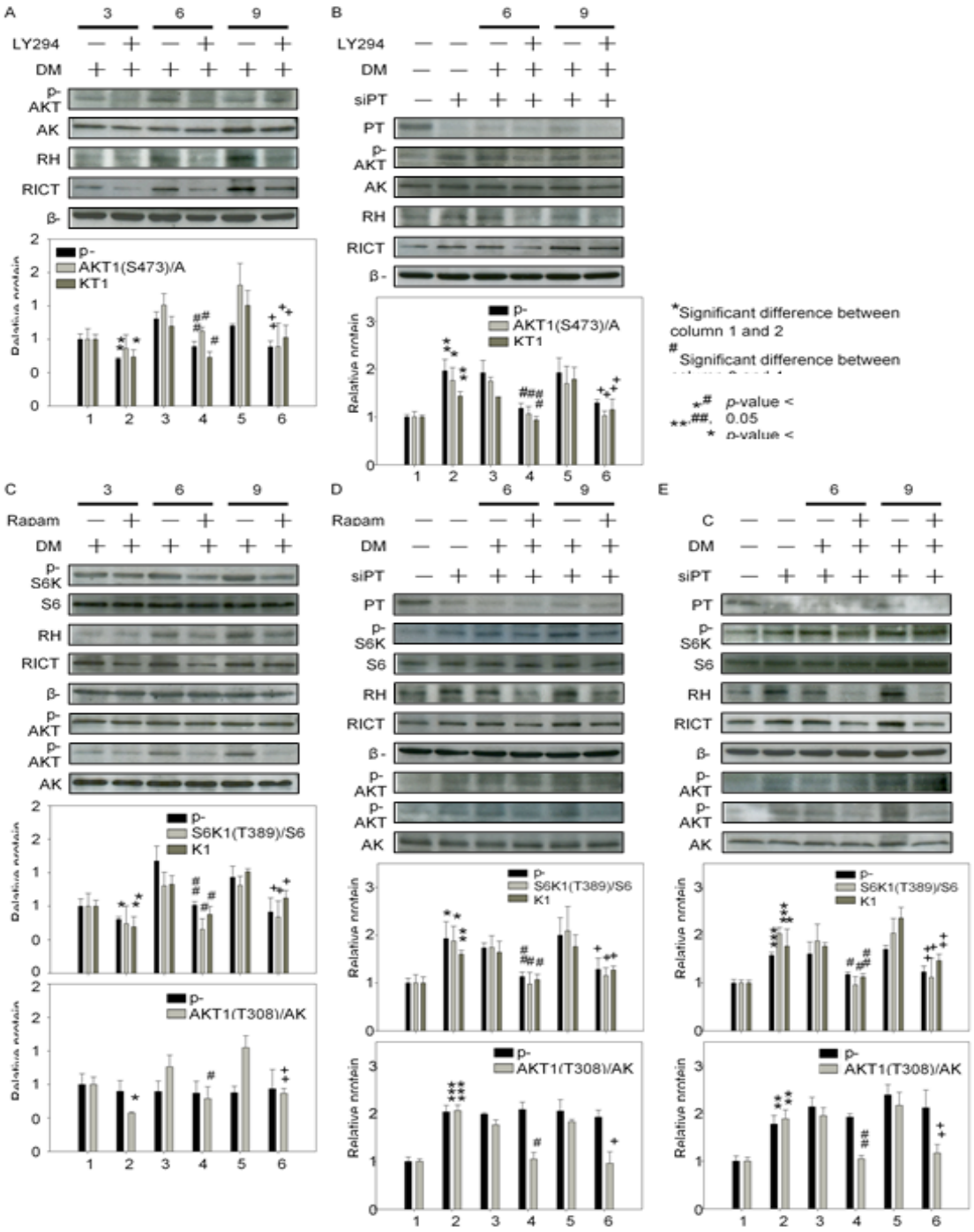
mRNA level of RHEB was similar in PC-3 and DU 145 cells, and PTEN-knockdown in DU 145 cells did not apparently enhance the expression of the RHEB mRNA (Figure 1D). These data indicated that the expression of PTEN and RHEB were negatively associated in only protein level.



Figures 1(A-D): Relationship between the expression levels of PTEN and RHEB. (A) PTEN and RHEB protein levels in 5 pairs of normal prostatic epithelia (N) and prostate tumors (T) were detected. GADPH served as the loading control. Quantified protein expressions were illustrated as levels relative to that in normal prostatic epithelia. (B) Protein levels of PTEN, RHEB, phosphorylated AKT1 (p-AKT1) on T308 and S473 in PC-3 and DU 145 cell lines were detected. β-actin served as the loading control. (C) Relative protein levels of PTEN, RHEB, RICTOR, total AKT1, p-AKT1 on T308 and S473 in DU 145 cells transfected with non-specific siRNA or PTEN-specific siRNA (siPTEN) were displayed. β-actin served as the loading control. Quantified protein expressions were exhibited as levels relative to that in cells transfected with non-specific siRNA. (D) The RHEB mRNA levels in PC-3 and DU 145 cells (left panel) and in DU 145 cells transfected with or without siPTEN (right panel) were detected. The RHEB mRNA levels were normalized by the β-actin mRNA levels. Columns and error bars indicated mean ± standard deviation.

As the major target of PTEN, the PI3K/AKT1 signaling was likely associated with the negative relationship between PTEN and RHEB. The PTEN inhibition on the PI3K/AKT1 signaling was simulated by the effect of LY294002. The level of p-AKT1 on S473 was lowered in the PC-3 cells treated with LY294002. Concomitant with the inactivated AKT1, the RHEB protein level was deceased in the treatments for 6 and 9 hours (Figure 2A). DU 145 cells were transfected with PTEN-specific siRNA and then treated with LY294002. The upregulated AKT1 phosphorylation and RHEB protein responding to PTEN-knockdown (Figure 2B, lane 1-2) were neutralized by LY294002 (Figure 2B, lane 3-6). The RHEB protein levels responding to PTEN knockdown and LY294002 treatment revealed that PTEN antagonizes the PI3K/AKT1 signaling to suppress the expression of the RHEB protein.

The negative association between PTEN and RHEB was identified only in protein level, and therefore we focused on the post-transcriptional control to elucidate the regulatory mechanism of PTEN on the RHEB expression. The expression of the RICTOR (rapamycin-insensitive companion of mTOR) protein is upregulated by the mTORC1/S6K1 (ribosomal protein S6 kinase 1)-mediated protein translation20. Similar changes of the RHEB and RICTOR protein levels responding to PTEN-knockdown and PI3K inhibition were noticed (Figure 1C and 2A-B). It was speculated that the expression of RHEB and RICTOR was controlled by PTEN through the identical mechanism. The mTORC1/S6K1 signaling was inhibited by rapamycin and manifested by the decreased level of p-S6K1(T389). As a validation of our speculation, inhibition of mTORC1 by rapamycin reduced the protein levels of RHEB and RICTOR in PC-3 cells. It was noticed that the p-AKT1(S473) level, but not the p-AKT1(T308) level, was diminished by rapamycin (Figure 2C). The increased levels of p-S6K1, RHEB, RICTOR and p-AKT1(S473) caused by PTEN-knockdown (Figure 2D, lane 1-2) were counteracted by rapamycin in DU 145 cells (Figure 2D, lane 3-6). Consistently, the elevated protein levels of RHEB and RICTOR resulting from PTEN knockdown in DU 145 cells (Figure 2E, lane 1-2) was abrogated by cycloheximide-induced restriction of protein translation (Figure 2E, lane 3-6). These results demonstrated that PTEN suppresses RHEB expression through inhibiting mTORC1/S6K1-mediated protein translation.



Figures 2(A-E): Signal pathways involved in the PTEN suppression on the RHEB protein expression. Protein levels in the following cells were detected. (A) PC-3 cells were treated with 1% DMSO or 25 μM of LY294002 for 3, 6 or 9 hours. (B) DU 145 cells were transfected with non-specific siRNA or PTEN-specific siRNA (siPTEN) for 48 hrs followed by incubation with 1% DMSO or 25 μM of LY294002 for another 6 or 9 hrs. (C) PC-3 cells were incubated with 1% DMSO or 50 μM of rapamycin for 3, 6 or 9 hrs. (D) DU 145 cells were transfected with non-specific siRNA or siPTEN for 48 hrs with following treatment with 1% DMSO or 50 μM of rapamycin for another 6 or 9 hrs. (E) DU 145 cells were transfected with non-specific siRNA or siPTEN for 48 hrs and then treated with 1% DMSO or 100 μg/ml cycloheximide for another 6 or 9 hrs. β-actin served as the loading control. Quantified protein expressions were displayed as levels relative to column 1. Columns and error bars indicated mean ± standard deviation.

Discussion

The overexpression of the RHEB protein in prostate cancer cells is the consequence of multiple events. The importance of the amplified RHEB during carcinogenesis has been noticed and emphasized for years [8,9,12]. However, little breakthrough has been made on the mechanism leading to this phenomenon. In this study, loss-of-PTEN, which is a well-known abnormality in prostate cancer, was linked for the first time to the increased RHEB protein in prostate cancer cells.

The higher rate of the PTEN loss and RHEB increase in more aggressive prostate tumors implies that the PTEN expression is likely involved in the RHEB expression [1,8]. PTEN was demonstrated to downregulate the expression of the RHEB protein in cell model. Consistently, we found that the PTEN protein decline is accompanied with the RHEB protein accumulation in prostate tumors. However, the upregulation of the RHEB expression also results from the genomic abnormalities and is unlikely associated with the function of PTEN [9]. It explained the observation that the RHEB protein increases in a tumor sample with regular protein level of PTEN.

PTEN inhibits the translation of oncoprotein in prostate cancer cells. The precursor synthesis of the IGF-1R (type 1 insulin-like growth factor receptor) protein is decreased in PTEN-overexpressing cells without the influence on the IGF-1R mRNA [7]. However, the PTEN downregulation on the IGF-1R protein synthesis is delineated as an AKT1-independent mechanism [7]. Our findings supported the AKT1-based pathway of PTEN on protein translation. It remains unknown that how does PTEN make the choice.

The mTORC1/S6K1-activated protein translation accounts for several positive feedback pathways [20]. The mTORC1/S6K1 pathway is stimulated by α_2-Macroglobulin in prostate cancer cells to elevate the protein levels of RAPTOR (regulatory-associated protein of mTOR) and RICTOR20. They are components of the mTORC1 [21] and mTORC222 complexes, respectively. The α_2-Macroglobulin-facilitated expression of RAPTOR reveals an mTORC1-mediated positive feedback regulation on itself [20]. In this study, the mTORC1/S6K1-enhanced RHEB expression is a positive feedback as well. The mTORC2 complex activates AKT1 through inducing the phosphorylation on S473 [23]. The diminished RICTOR protein level led by rapamycin or cycloheximide in both cell lines attenuates the level of p-AKT1(S473) instead of p-AKT1(T308). The PTEN suppression on protein translation interrupts the positive feedback on the RHEB protein expression and the AKT1 activity.

Conclusions

We demonstrated that the expression of the RHEB protein is downregulated by PTEN through suppressing the protein translation mediated by the PI3K/AKT1/mTORC1/S6K1 kinase cascade. The loss of PTEN, which frequently occurs in prostate cancer cells, could therefore benefit the upregulation of the RHEB protein level. This study reported for the first time a PTEN-involved post-transcriptional control on the RHEB expression and provided an explanation for the prostate cancers expressing the elevated RHEB expression in only protein level. However, there might be other post-transcriptional mechanisms for regulating the RHEB expression in prostate cancer, and further investigations are needed to explore them.

Acknowledgement: This study was supported by research grants from Chang Gung Memorial Hospital.

References

1. Murphy SJ, Karnes RJ, Kosari F, Castellar BE, Kipp BR, et al. (2016) Integrated analysis of the genomic instability of PTEN in clinically insignificant and significant prostate cancer. Mod Pathol 29: 143-156.
2. Gravina GL, Biordi L, Martella F, et al. (2009) Epigenetic modulation of PTEN expression during antiandrogenic therapies in human prostate cancer. Int J Oncol 35: 1133-1139.
3. Li J, Yang X, Guan H, Flati V, Ricevuto E, et al. (2016) Exosome-derived microRNAs contribute to prostate cancer chemoresistance. Int J Oncol 49: 838-846.
4. Vanhaesebroeck B, Stephens L, Hawkins P (2012) PI3K signalling: the path to discovery and understanding. Nat Rev Mol Cell Biol 13: 195-203.
5. Lim HJ, Crowe P, Yang JL (2015) Current clinical regulation of PI3K/PTEN/Akt/mTOR signalling in treatment of human cancer. J Cancer Res Clin Oncol 141: 671-689.
6. Sun Y, Tian H, Wang L (2015) Effects of PTEN on the proliferation and apoptosis of colorectal cancer cells via the phosphoinositol-3-kinase/Akt pathway. Oncol Rep 33: 1828-1836.
7. Zhao H, Dupont J, Yakar S, Karas M, LeRoith D, et al. (2004) PTEN inhibits cell proliferation and induces apoptosis by downregulating cell surface IGF-IR expression in prostate cancer cells. Oncogene 23: 786-794.
8. Kobayashi T, Shimizu Y, Terada N, Yamasaki T, Nakamura E, et al. (2010) Regulation of androgen receptor transactivity and mTOR-S6 kinase pathway by Rheb in prostate cancer cell proliferation. Prostate 70: 866-874.
9. Nardella C, Chen Z, Salmena L, Carracedo A, Alimonti A, et al. (2008) Aberrant Rheb-mediated mTORC1 activation and Pten haploinsufficiency are cooperative oncogenic events. Genes Dev 22: 2172-2177.
10. Chakraborty S, Mohiyuddin SM, Gopinath KS, Kumar A (2008) Involvement of TSC genes and differential expression of other members of the mTOR signaling pathway in oral squamous cell carcinoma. BMC Cancer 8: 163.
11. Zheng H, Liu A, Liu B, Li M, Yu H, et al. (2010) Ras homologue enriched in brain is a critical target of farnesyltransferase inhibitors in non-small cell lung cancer cells. Cancer Lett 297: 117-125.
12. Lu ZH, Shvartsman MB, Lee AY, Shao JM, Murray MM, et al. (2010) Mammalian target of rapamycin activator RHEB is frequently overexpressed in human carcinomas and is critical and sufficient for skin epithelial carcinogenesis. Cancer Res 70: 3287-3298.

13. Groenewoud MJ, Zwartkruis FJ (2013) Rheb and Rags come together at the lysosome to activate mTORC1. Biochem Soc Trans 41: 951-955.

14. Garrido A, Brandt M, Djouder N (2016) Transport to Rhebpress activity. Small GTPases 7: 12-15.

15. Ghosh AP, Marshall CB, Coric T, Shim FH, Kirkman R, et al. (2015) Point mutations of the mTOR-RHEB pathway in renal cell carcinoma. Oncotarget 6: 17895-17910.

16. Carroll B, Maetzel D, Maddocks OD, Otten G, Ratcliff M, et al. (2016) Control of TSC2-Rheb signaling axis by arginine regulates mTORC1 activity. Elife 5: 11058.

17. Dibble CC, Cantley LC (2015) Regulation of mTORC1 by PI3K signaling. Trends Cell Biol 25: 545-555.

18. Hall DJ, Grewal SS, de la Cruz AF, et al. (2007) Rheb-TOR signaling promotes protein synthesis, but not glucose or amino acid import, in Drosophila. BMC Biol 5: 10.

19. Wang Y, Huang BP, Luciani DS, Wang X, Johnson JD, et al. (2008) Rheb activates protein synthesis and growth in adult rat ventricular cardiomyocytes. J Mol Cell Cardiol 45: 812.

20. Misra UK, Pizzo SV (2012) Receptor-recognized alpha(2)-macroglobulin binds to cell surface-associated GRP78 and activates mTORC1 and mTORC2 signaling in prostate cancer cells. PLoS One 7: e51735.

21. Aghamohammadzadeh R, Zhang YY, Stephens TE, Arons E, Zaman P, et al. (2016) Up-regulation of the mammalian target of rapamycin complex 1 subunit Raptor by aldosterone induces abnormal pulmonary artery smooth muscle cell survival patterns to promote pulmonary arterial hypertension. FASEB J 30: 2511.

22. Cui Y, Zhao J, Yi L, Jiang Y (2016) microRNA-153 Targets mTORC2 Component Rictor to Inhibit Glioma Cells. PLoS One 11: e0156915.

23. Salazar M, Lorente M, Garcia-Taboada E, Gómez EP, Dávila D, et al. (2015) TRIB3 suppresses tumorigenesis by controlling mTORC2/AKT/FOXO signaling. Mol Cell Oncol 2: e980134.