

2014

# Renin angiotensin system modulates mTOR pathway through AT2R in HIVAN

P. Rai

*Northwell Health*

R. Lederman

*Northwell Health*

S. Hague

*Northwell Health*

S. Rehman

*Northwell Health*

V. Kumar

*Northwell Health**See next page for additional authors*Follow this and additional works at: <https://academicworks.medicine.hofstra.edu/articles>Part of the [Nephrology Commons](#)

## Recommended Citation

Rai P, Lederman R, Hague S, Rehman S, Kumar V, Sataranatrajan K, Malhotra A, Kasinath B, Singhal P. Renin angiotensin system modulates mTOR pathway through AT2R in HIVAN. . 2014 Jan 01; 96(3):Article 2441 [ p.]. Available from: <https://academicworks.medicine.hofstra.edu/articles/2441>. Free full text article.

This Article is brought to you for free and open access by Donald and Barbara Zucker School of Medicine Academic Works. It has been accepted for inclusion in Journal Articles by an authorized administrator of Donald and Barbara Zucker School of Medicine Academic Works. For more information, please contact [academicworks@hofstra.edu](mailto:academicworks@hofstra.edu).

---

**Authors**

P. Rai, R. Lederman, S. Hague, S. Rehman, V. Kumar, K. Sataranatrajan, A. Malhotra, B. S. Kasinath, and P. C. Singhal

Published in final edited form as:

*Exp Mol Pathol.* 2014 June ; 96(3): 431–437. doi:10.1016/j.yexmp.2014.04.004.

## Renin angiotensin system modulates mTOR pathway through AT2R in HIVAN

Partab Rai<sup>a</sup>, Rivka Lederman<sup>a</sup>, Shabirul Haque<sup>a</sup>, Shabina Rehman<sup>a</sup>, Viki Kumar<sup>a</sup>, Kavithalakshmi Sataranatrajan<sup>b</sup>, Ashwani Malhotra<sup>a</sup>, Balakuntalam S. Kasinath<sup>b</sup>, and Pravin C. Singhal<sup>a,\*</sup>

Pravin C. Singhal: psinghal@nshs.edu

<sup>a</sup>Renal Molecular Research Laboratory, Feinstein Institute for Medical Research, Hofstra North Shore LIJ Medical School, New York, NY, United States

<sup>b</sup>University of Texas Health Science Center, San Antonio, TX, United States

### Abstract

Mammalian target of rapamycin (mTOR) has been reported to contribute to the development of HIV-associated nephropathy (HIVAN). We hypothesized that HIV may be activating renal tissue mTOR pathway through renin angiotensin system (RAS) via Angiotensin Receptor Type II receptor (AT2R). Renal tissues of Vpr transgenic and Tg26 (HIVAN) mice displayed enhanced phosphorylation of mTOR and p70S6K. Aliskiren, a renin inhibitor attenuated phosphorylation of both mTOR and p70S6K in renal tissues of HIVAN mice. Interestingly, Angiotensin Receptor Type I (AT1R) blockade did not modulate renal tissue phosphorylation of mTOR in HIVAN mice; on the other hand, AT2R blockade attenuated renal tissue phosphorylation of mTOR in HIVAN mice. In vitro studies, both renin and Ang II displayed enhanced mouse tubular cell (MTC) phosphorylation of p70S6K in a dose dependent manner. HIV/MTC also displayed enhanced phosphorylation of both mTOR and p70S6K; interestingly this effect of HIV was further enhanced by losartan (an AT1R blocker). On the other hand, AT2R blockade attenuated HIV-induced tubular cell phosphorylation of mTOR and p70S6K, whereas, AT2R agonist enhanced phosphorylation of mTOR and p70S6K. These findings indicate that HIV stimulates mTOR pathway in HIVAN through the activation of renin angiotensin system via AT2R.

### Keywords

Renal proximal tubular cells; Angiotensin II type I receptor; Angiotensin II type II receptor; Renin

### Introduction

The activation of renin angiotensin system (RAS) has been demonstrated to play an important role in the development as well as progression of HIVAN (Kumar et al., 2011,

2012). Modalities which either blocked the production of Ang II or inhibited the effect of Ang II have been reported to slow down the progression of HIVAN (Bird et al., 1989; Burns et al., 1999; Kimmel et al., 2003); moreover, infusion of Ang II accelerated the progression of renal lesions in a mouse model of HIVAN (Ideura et al., 2007). Recently, mTOR pathway has been demonstrated to play an important role in the development of proliferative HIVAN phenotype in HIVAN (Kumar et al., 2010; Rai et al., 2013; Rehman et al., 2012). We hypothesized that renin angiotensin system may be contributing to the activation of mTOR pathway in HIVAN.

Ang II exerts its effects through its receptors AT<sub>1</sub>R and AT<sub>2</sub>R (de P Rodrigues et al., 2006). The AT<sub>1</sub>R participates predominantly in the induction of proliferation, hypertension, coagulation, and inflammation (Griendling et al., 1996); whereas, the activation of AT<sub>2</sub>R induces opposite effects – apoptosis, hypotension, and anti-inflammatory events (Griendling et al., 1996; Timmermans et al., 1993). On that account, blockade of AT<sub>1</sub>R may display effects as of the activation of AT<sub>2</sub>R and vice versa. We have recently reported that during the blockade of AT<sub>1</sub>R, renal tissue AT<sub>2</sub>R was up regulated in HIVAN (Salhan et al., 2012a). These studies suggested that the activation of AT<sub>2</sub>R during the blockade of AT<sub>1</sub>R also contributed to the amelioration of renal lesions in HIVAN.

HIVAN is characterized by kidney cell proliferative phenotype in the form of collapsing glomerulopathy and microcystic dilatation of tubules (Atta, 2010). Entry of podocytes into proliferative phenotype has been attributed to the effect of HIV on podocytes leading to their re-entry into cell cycle (Barisoni et al., 1999), activation of mTOR pathway (Kumar et al., 2010), and induction of epithelial mesenchymal transition (Yadav et al., 2010). Ang II is a known inducer of epithelial mesenchymal transition in kidney cells (Mezzano et al., 2001; Wolf, 2006). Similarly, Ang II has also been reported to activate mTOR pathway (Yoshida et al., 2013). However, this effect of Ang II has been predominantly mediated through AT<sub>1</sub>R in several cell types (Burks et al., 2011; Diniz et al., 2009; Mavroei et al., 2013). The role of AT<sub>2</sub>R in mTOR signaling in HIVAN has not been studied to date.

In the present study, we evaluated the role of renin angiotensin system (RAS) in the activation of mTOR pathway in HIVAN. We have used two mouse models of HIVAN to study the role of Ang II receptors.

## Material and methods

### HIV transgenic mice

We have used age and sex matched FVB/N (control), Vpr transgenic and Tg26 (on FVB/N background). Breeding pairs of FVB/N were obtained from Jackson Laboratories (Bar Harbor, ME). Breeding pairs to develop Vpr colonies were kindly gifted by Prof. Jeffery B Kopp, M.D., National Institutes of Health, Bethesda, MD. Breeding pairs to develop Tg26 colonies were kindly gifted by Prof. Paul E. Klotman M.D., President and CEO, Baylor College of Medicine, Houston, TX. Details of these mice have been described previously (Kumar et al., 2010, 2011, 2012; Rai et al., 2013). We are maintaining the colonies of these animals in our animal facility. The Ethics Review Committee for Animal Experimentation of Long Island Jewish Medical Center approved the experimental protocol.

### Proximal tubular cells

Mouse proximal tubular epithelial cells (MTCs) were gift from Dr. G. Wolf (Department of Medicine, Hamburg, Germany). These cells are well characterized and expressed tubular cell markers (Rehman et al., 2012).

### Experimental studies

We have generated *Vpr* transgenic animals by crossing podocin/*rtTA* (constitutively expresses the *rtTA* which is a fusion protein comprised of the TetR repressor and the VP16 transactivation domain expressed under control of the podocin promoter) mice with tetop/*Vpr* mice (TRE-regulated *Vpr* gene). These animals were fed doxycycline in their drinking water to induce the expression of the podocyte specific *Vpr* gene. These animals develop apparent HIVAN phenotype only after six weeks of doxycycline administration.

### Protocol A

Control (FVB/N) and *Vpr* transgenic mice (4 week old) were fed doxycycline in drinking water for six weeks (n = 6). Subsequently, mice were sacrificed and evaluated for renal tissue protein(s) expression by Western blotting. Additionally, renal tissues of 4 week old control and Tg26 mice (n = 4) were harvested for Western blotting studies.

### Protocol B

Four week old *Vpr* mice in groups of six were administered either saline (S), Doxy (in drinking water), or Doxy + aliskiren (50 mg/kg by miniosmotic pumps) for six weeks. Subsequently, animals were sacrificed and kidneys were harvested and cellular lysates were prepared for Western blotting analysis.

### Protocol C

Twelve Tg26 mice aging 3 weeks in groups of four were anesthetized by inhalation anesthesia (isoflurane + oxygen). The Alzet minipumps (model # 2004, Durect Corp. Cupertino, CA) containing either saline or Telmisartan (AT<sub>1</sub>R blocker, 300 µg/day), or PD123319 (AT<sub>2</sub>R blocker, 3 µg/day) were implanted subcutaneously. Four age and sex matched FVBN mice – receiving saline through minipumps – served as control for Tg26 mice receiving saline. After two weeks of infusion, animals were sacrificed and Western blotting studies were carried out from the renal tissues. Renal cortical sections were evaluated for immunohistochemical studies.

### Immunohistochemical staining

The immunohistochemistry protocol used in the present study has been described previously (Kumar et al., 2010). The primary antibody -phospho-mTOR (1:500, #2971, Cell Signaling Technology Inc., Danvers, MA) - was followed by the secondary antibody at 1:250 dilution and then incubated in ABC reagent (Vector Laboratories, Burlingame, CA) for 30 min. Sections were washed 3× in PBS buffer and placed in VECTOR Nova RED substrate kit SK-4800 (Vector Laboratories, Burlingame, CA) followed by counterstaining with methyl green.

## Western blotting studies

Renal tissues of control and experimental animals and cells (control and HIV-transduced, under control and experimental conditions) were lysed in RIPA buffer containing 50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% Deoxycholate, 0.1% SDS, 1 × protease inhibitor cocktail (Calbiochem, Cocktail Set I), 1 mM PMSF, and 0.2 mM sodium orthovanadate. Protein concentration was measured with the BioRad Protein Assay kit (BioRad Labs). Total protein extracts (20–30 µg/ml) were separated on a 4–15% polyacrylamide (PAGE) gel (Bio-Rad, Hercules, CA) and transferred onto a nitrocellulose membrane using Bio-Rad miniblott apparatus. The blots were blocked with 0.5% BSA and 0.1% TWEEN 20 in 1 × PBS for 60 min at room temperature and then hybridized with the anti-phospho-mTOR (1:500, Cell Signaling Technology Inc., Danvers, MA), anti-phospho-p70S6K (1:500, Cell Signaling), anti-phospho-eEF2 (1:500, rabbit polyclonal, Cell Signaling) and β-laminin (Santa Cruz) antibodies and subsequently treated with horseradish peroxidase labeled appropriate secondary antibodies. The blots were developed using a chemiluminescence detection kit (PIERCE, Rockford, IL) and exposed to X-ray film (Eastman Kodak Co., Rochester, NY). Equal protein loading and the protein transfer were confirmed by stripping and immunoblotting for actin protein using a polyclonal α-Actin antibody (I-19, Santa Cruz) on the same Western blots.

## Statistical analysis

For comparison of mean values between two groups, the unpaired t test was used. To compare values between multiple groups, analysis of variance (ANOVA) was applied and a Bonferroni multiple range test was used to calculate a P-value. Statistical significance was defined as  $P < 0.05$ .

## Results

### Vpr mice display activation of mTOR pathway in renal tissues

Activation of mTOR has been previously reported in Tg26 mice (Kumar et al., 2010). In the present study we studied the activation of mTOR pathway in another model of HIVAN, Vpr transgenic mice. Protein blots of renal tissues of control and Vpr mice ( $n = 4$ ) were prepared and probed for phospho-mTOR and downstream molecules. Representative gels of two mice are shown (Fig. 1). Immunoblots of Vpr mice showed increase in Ser<sup>2448</sup> phos of mTOR and in Thr<sup>389</sup> phos of p70S6 kinase; both suggest activation of mTOR in the renal tissues. Moreover, reduction in Eukaryotic Elongation Factor (eEF2) phos of Thr<sup>56</sup> is indicative of increase in p70S6 kinase activation and stimulation of elongation phase of mRNA translation. In addition, laminin β1 content appears to be increased in the renal tissues of Vpr mice. Thus, Vpr mice show evidence of mTOR activation and stimulation of elongation phase of mRNA translation. Renal tissues of Tg26 mice also displayed activation of mTOR pathway (data not shown).

### Renin inhibition attenuates renal tissue mTOR phosphorylation and downstream signaling

To determine the effect of renin on renal tissue mTOR phosphorylation control, renal tissues of saline receiving, Doxy-receiving, and Doxy/Alis receiving mice were electrophoresed and

protein blots were probed for phospho-mTOR and phospho-p70S6K expressions. The same blots were reprobbed for total mTOR and actin. Representative lysates for two animals are shown. Vpr mice displayed enhanced phosphorylation of both mTOR and p70S6K (Fig. 2); whereas doxy/Alis mice displayed attenuated renal tissue phosphorylation of both mTOR as well as p70S6K. These findings indicate that renin inhibition not only inhibited phosphorylation of mTOR but also attenuated activation of the mTOR pathway.

### **AT1R blockade enhances whereas AT2R blockade attenuates renal tissue mTOR phosphorylation in HIVAN mice**

To determine the effect of AT1R blockade on kidney cell mTOR phosphorylation, renal cortical sections of control, Tg26, and Tg26 mice receiving telmisartan (Tg26/Tel) were immunolabeled for phospho-mTOR. Representative microphotographs are shown in Fig. 3A. Both podocytes and tubular cells displayed enhanced phosphorylation of mTOR in Tg26 mice. However, kidney cells of Tg26/Tel mice displayed phosphorylation of mTOR predominantly in their nuclei.

In parallel sets of experiments, protein blots of renal tissues of control, Tg26, and Tg26/Tel ( $n = 4$ ) were probed for phospho-mTOR. The same blots were reprobbed for actin. Representative gels are shown in Fig. 3B. Cumulative data for 4 sets of experiments are shown in bar graphs. HIV enhanced ( $P < 0.01$ ) expression of renal tissue phosphorylation of mTOR in Tg26 mice. However, AT1R blockade further enhanced ( $P < 0.01$ ) this effect of HIV.

To determine the effect of AT2R blockade of renal tissue phosphorylation of mTOR in HIVAN mice, protein blots of control, Tg26, and Tg26-receiving AT2B were probed for phospho-mTOR ( $n = 4$ ). The same blots were reprobbed for actin. Representative gels are shown in Fig. 3C. Cumulative data are shown in bar graphs. Tg26 mice displayed enhanced renal tissue phosphorylation of mTOR; however, renal tissues of Tg26/AT2B mice displayed attenuate expression of phospho-mTOR. These findings indicate that HIV-induced renal tissue phosphorylation is mediated via AT2R.

### **In vitro studies**

**Renin enhances tubular cell mTOR activation**—We have previously reported that HIV not only phosphorylated mTOR but also activated mTOR pathway in tubular cells (Rehman et al., 2012). Additionally, we reported that HIV stimulated tubular cell renin production through down regulation of VDR (Chandel et al., 2013; Salhan et al., 2012b). Thus, it is plausible that HIV activates tubular cell mTOR pathway via tubular cell generation of renin.

To establish a causal relationship between renin and the activation of tubular cell mTOR, we evaluated the effect of aliskiren on HIV-induced tubular cell activation of mTOR pathway. EV/MTCs and HIV/MTCs were incubated in media containing either buffer or aliskiren ( $10^{-6}$  M) for 24 h ( $n = 3$ ). Subsequently, protein blots were probed for phospho-p70S6K and the same blots were reprobbed for actin. Representative gels of two different cellular lysates are displayed in Fig. 4A. cumulative data are shown in a bar diagram. HIV/MTCs displayed

higher expression of phospho-p70S6K when compared to EV/MTCs. Aliskiren attenuated tubular cell phosphorylation of p70S6K under control and HIV stimulated states. These findings indicate that renin inhibition is associated with down regulation of tubular cell mTOR pathway in control as well as in HIV milieu.

To determine the dose response effect of renin on tubular cell phosphorylation of p70S6K, MTCs were incubated in media containing either buffer or variable concentrations of renin (0, 1, 10, 100, and 1000 pM) for 24 h. Subsequently protein blots were prepared and probed for phospho-p70S6K. The same blots were reprobed for actin. Representative gels are shown in Fig. 4B. Cumulative data are shown in the form of bar graphs. Renin enhanced tubular cell phosphorylation of p70S6K in a dose dependent manner. These findings indicate that renin has a potential to stimulate mTOR pathway in tubular cells.

**AT1R blockade induces mTOR activation under control and HIV milieu**—Since tubular cells express angiotensinogen, it could be possible that treatment of tubular cells with renin might have enhanced the generation of Ang II; the latter might have activated tubular cell mTOR pathway. To exclude the role of Ang II in HIV induced activation of the mTOR pathway, EV/MTC and HIV/MTC were incubated in media containing either buffer or losartan ( $10^{-7}$  M) for 24 h. Cellular lysates were electrophoresed and probed for phospho-mTOR. The same blots were reprobed for actin. Representative gels from two different cell lysates are shown in Fig. 4C. Cumulative data ( $n = 3$ ) are shown in the form of a bar diagram. Losartan enhanced mTOR phosphorylation in EV/MTCs as well as in HIV/MTCs.

To determine the activation of mTOR downstream signaling, EV/MTC and HIV/MTC were incubated in media containing either buffer or losartan ( $10^{-7}$  M) for 24 h ( $n = 3$ ) Protein blots were probed for phospho-p70S6K. The same blots were reprobed for actin. Representative gels from two different cell lysates are shown in Fig. 4D. Cumulative data ( $n = 3$ ) are shown in the form of a bar diagram. Losartan enhanced phosphorylation of p70S6K in EV/MTCs as well as in HIV/MTCs. These findings confirmed that AT1R blockade enhances activation of mTOR pathway in tubular cells.

To determine the effect of ARBs on tubular cell renin expression by MTCs, EV/MTCs and HIV/MTCs were incubated in media containing either buffer or losartan ( $10^{-7}$  M) for 24 h. Subsequently, protein blots were probed for renin and reprobed for actin. Representative gels from two different sets of experiments are shown in Fig. 5. Cumulative densitometric data ( $n = 3$ ) are shown in bar graphs. HIV/MTC displayed higher expression of renin when compared to EV//MTC. Losartan enhanced tubular cell renin expression both under control and HIV milieu. These findings suggested that losartan induced tubular cell phosphorylation of p70S6K might have mediated through tubular cell renin generation.

**Ang II enhances tubular cell mTOR phosphorylation**—To determine the effect of Ang II on tubular mTOR phosphorylation, EV/MTCs and HIV/MTCs were incubated in media containing variable concentrations of Ang II (0 to  $10^{-6}$  M) for 24 h ( $n = 3$ ). Subsequently, protein blots were probed for phospho-p70S6K. The same blots were reprobed for actin. Representative gels are shown in Fig. 6. Cumulative densitometric data

are shown in bar graphs. Ang II enhanced tubular cell p70S6K phosphorylation in a dose dependent manner.

#### **AT2R blockade attenuate HIV-induced activation of tubular cell mTOR**

**pathway**—To determine the role of AT2R, EV/MTCs and HIV/MTCs were incubated in media containing either buffer or AT2R blocker (PD123319, 1  $\mu$ M) for 24 h (n = 3). Protein blots were probed for phospho-mTOR and reprobbed for actin. Representative gels are shown in Fig. 7A. Cumulative data are shown in bar graphs. AT2R blockade inhibited HIV-induced tubular cell mTOR phosphorylation. These findings indicated that HIV-induced tubular cell mTOR phosphorylation was mediated through AT2R.

To study the effect of AT2R, on tubular cell mTOR downstream signaling, EV/MTCs and HIV/MTCs were incubated in media containing either buffer or PD123319 (1  $\mu$ M) for 24 h (n = 3). Protein blots were probed for phospho-p70S6K and reprobbed for actin. Representative gels are shown in Fig. 7B. Cumulative data are shown in bar graphs. AT2R blockade inhibited HIV-induced tubular cell p70S6k phosphorylation. These findings indicated that AT2R blocked also inhibited HIV-induced tubular cell mTOR downstream signaling.

#### **AT2 agonist (AT2A) enhance tubular cell phosphorylation of p70S6K**

—To determine the effect of AT2A on tubular cell mTOR pathway activation, EV/MTCs and HIV/MTCs were incubated in media containing either buffer or AT2A (CGP42112, 1  $\mu$ M) for 24 h (n = 3). Protein blots were probed for phospho-p70S6K and reprobbed for actin. Representative gels are shown in Fig. 8. Cumulative data are shown in bar graphs. Both HIV and AT2A enhanced tubular cell phosphorylation of p70S6K. However, AT2A did not exacerbate HIV-induced tubular phosphorylation of p70S6K. We speculate that HIV stimulated tubular cell phosphorylation reached its maximum and therefore AT2A could not modulate it further.

## **Discussion**

In the present study, renal tissues of Vpr and HIV transgenic mice displayed enhanced phosphorylation of mTOR and p70S6K. Since aliskiren inhibited renal tissue mTOR activation, it suggested that HIV-induced renal tissue renin generation might have contributed to it. However, AT1 blockade enhanced renal tissue phosphorylation of p70S6K; whereas, AT2 blockade attenuated renal tissue phosphorylation of p70S6K. These findings indicated that HIV-induced renal tissue mTOR activation was mediated through AT2R. In vitro studies, HIV/MTC displayed enhanced phosphorylation of p70S6K, however, this effect of HIV was also attenuated by aliskiren. Both renin and Ang II displayed enhanced tubular cell phosphorylation of p70S6K in a dose dependent manner; however, AT1 blockade enhanced HIV-induced tubular cell phosphorylation of p70S6K. On the other hand, AT2 blockade attenuated HIV-induced tubular cell phosphorylation of p70S6K, whereas, AT2 agonist (AT2A) enhanced phosphorylation of p70S6K. These findings confirmed that HIV induced tubular cell mTOR activation is mediated through AT2R.

AT<sub>2</sub>R expression is usually higher than AT<sub>1</sub>R expression during embryogenesis (Ciuffo et al., 1993; Shanmugam et al., 1995). However, this trend reverses after birth and continues at later time periods. AT<sub>2</sub>R is uniformly expressed in tubules and vascular segments but its expression varies in glomeruli (Cao et al., 2000; Ozono et al., 1947; Ruiz-Ortega et al., 2003). These discrepancies have been attributed to species differences (Armando et al., 2002; Baiardi et al., 2005). We previously reported the activation of the RAS in HIVAN mice (Kumar et al., 2011; Kumar et al., 2012). In in vitro studies too, HIV enhanced the activation of the RAS in human podocytes as well as tubular cells (Chandel et al., 2013; Salhan et al., 2012b). This effect of HIV has been attributed to down regulation of liganded vitamin D receptors in HIV infected cells (Chandel et al., 2013; Salhan et al., 2012b). Although, adult kidney cells barely display any expression of AT<sub>2</sub>R but its expression increases in the models of overt renal damage, such as induced by renal ablation and subtotal nephrectomy, or kidney damage caused by protein overload (Bautista et al., 2001; Cao et al., 2002; Hashimoto et al., 2004; Ruiz-Ortega et al., 2003; Tejera et al., 2004; Vazquez et al., 2005). On the other hand, renal tissues in HIVAN mice have been reported to display attenuated expression of AT<sub>2</sub>R (Salhan et al., 2012a). However, blockade of AT<sub>1</sub>R enhanced renal tissue expression of AT<sub>2</sub>R in these studies.

Mavroei et al. studied the effect of losartan on mTOR pathway in a rat model of diabetic nephropathy (Mavroei et al., 2013). Untreated diabetic rats displayed enhanced activated forms of mTOR in glomeruli and podocytes. However, losartan-receiving diabetic rats displayed a decrease in both phosphorylated and activated forms of mTOR (Ser<sup>2448</sup>) in glomeruli only; whereas podocytes exhibited no change in phosphorylated mTOR. On the other hand, glomeruli and podocytes of non-diabetic animals displayed an inverse pattern. In Ren2 rats, a transgenic RAS model, blockade of AT<sub>1</sub>R led to inhibition of total and Ser<sup>2448</sup> phosphorylated mTOR (Whaley-Connell et al., 2011). These reports supported a role of AT<sub>1</sub>R receptor in mTOR/S6K1 signaling in the kidney. On the other hand, acute hyperglycemia enhanced activation of tubular cell mTOR pathway in diabetic mice through AT<sub>2</sub>R rather than through AT<sub>1</sub>R (Day et al., 2010). These investigators demonstrated that hyperglycemia stimulated Ang II generation, which activated both AT<sub>1</sub>R and AT<sub>2</sub>R; however, only blockade of AT<sub>2</sub>R prevented the activation of mTOR pathway; moreover, hyperglycemia did not activate kidney cell mTOR pathway in AT<sub>2</sub>R knockout mice. Thus, these investigators highlighted the role of AT<sub>2</sub>R in the activation of mTOR pathway in acute hyperglycemia. Our findings in the HIVAN mice are consistent with the observations of these investigators.

In the present study, both renin and Ang II enhanced activation of mTOR pathway in tubular cells. Renin inhibitor also inhibited activation of mTOR pathway in HIVAN mice as well as in tubular cells. However, it did not exclude the effect of Ang II on mTOR activation. Since AT<sub>1</sub> blockade displayed enhanced renal tissue expression of phospho-mTOR as well as tubular cell expression of phospho-mTOR, we considered that the possibility of activation of mTOR during AT<sub>1</sub>R blockade was mediated either through renin or through unopposed action of AT<sub>2</sub>R. Since blockade of AT<sub>2</sub>R in tubular cells attenuated the effect of Ang II on tubular cell mTOR activation, it confirmed that the Ang II-induced effect was mediated through AT<sub>2</sub>R.

In summary, HIV enhanced tubular cell Ang II production via down regulation of VDR and enhanced expression of renin. Ang II enhanced activation of tubular cell mTOR pathway via AT2R (Fig. 9).

## Acknowledgments

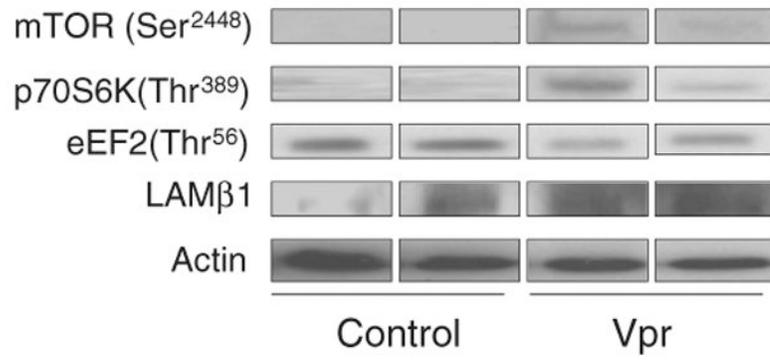
This work was supported by grants RO1DK084910, RO1 DK083931, and 1R01DK098074 (PCS) from the National Institutes of Health, Bethesda, MD.

## References

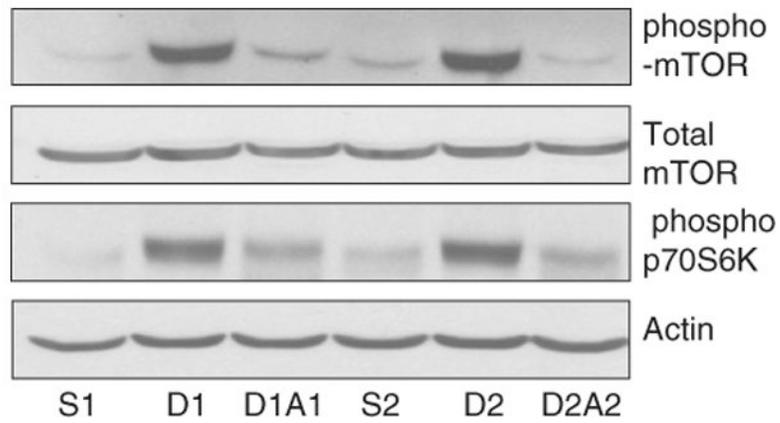
- Armando I, Jezova M, Juorio AV, Terron JA, Falcon-Neri A, Semino-Mora C, et al. Estrogen upregulates renal angiotensin II AT(2) receptors. *Am J Physiol Renal Physiol.* 2002; 283:F934–F943. [PubMed: 12372768]
- Atta MG. Diagnosis and natural history of HIV-associated nephropathy. *Adv Chronic Kidney Dis.* 2010; 17:52–58. [PubMed: 20005489]
- Baiardi G, Macova M, Armando I, Ando H, Tyurmin D, Saavedra JM. Estrogen upregulates renal angiotensin II AT1 and AT2 receptors in the rat. *Regul Pept.* 2005; 124:7–17. [PubMed: 15544836]
- Barisoni L, Kriz W, Mundel P, D'Agati V. The dysregulated podocyte phenotype: a novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol.* 1999; 10:51–56. [PubMed: 9890309]
- Bautista R, Sanchez A, Hernandez J, Oyekan A, Escalante B. Angiotensin II type AT(2) receptor mRNA expression and renal vasodilatation are increased in renal failure. *Hypertension.* 2001; 38:669–673. [PubMed: 11566953]
- Bird JE, Durham SK, Giancarli MR, Gitliz PH, Pandya DG, Dambach DM, Mozes MM, Kopp B. Captopril prevents nephropathy in HIV-transgenic mice. *J Am Soc Nephrol.* 1989; 9:1441–1447. [PubMed: 9697666]
- Burks TN, Andres-Mateos E, Marx R, Mejias R, Van Erp C, Simmers JL, Walston JD, Ward CW, Cohn RD. Losartan restores skeletal muscle remodeling and protects against disuse atrophy in sarcopenia. *Sci Transl Med.* 2011; 3:82.
- Burns C, Paul SK, Toth IR, Siva L. Effect of angiotensin-converting enzyme inhibition in HIV-associated nephropathy. *J Am Soc Nephrol.* 1999; 8:1140–1146. [PubMed: 9219164]
- Cao Z, Kelly DJ, Cox A, Casley D, Forbes JM, Martinello P, et al. Angiotensin type 2 receptor is expressed in the adult rat kidney and promotes cellular proliferation and apoptosis. *Kidney Int.* 2000; 58:2437–2451. [PubMed: 11115077]
- Cao Z, Bonnet F, Candido R, Nesteroff SP, Burns WC, Kawachi H, et al. Angiotensin type 2 receptor antagonism confers renal protection in a rat model of progressive renal injury. *J Am Soc Nephrol.* 2002; 13:1773–1787. [PubMed: 12089373]
- Chandel N, Sharma B, Husain M, Salhan D, Singh T, Rai P, Mathieson PW, Saleem MA, Malhotra A, Singhal PC. HIV compromises integrity of the podocyte actin cytoskeleton through downregulation of the vitamin D receptor. *Am J Physiol Renal Physiol.* 2013; 304:F1347–F1357. [PubMed: 23467424]
- Ciuffo GM, Viswanathan M, Seltzer AM, Tsutsumi K, Saavedra JM. Glomerular angiotensin II receptor subtypes during development of rat kidney. *Am J Physiol.* 1993; 265:F264–F271. [PubMed: 8368335]
- Day RT, Cavaglieri Rde C, Tabatabaie H, Mantravadi V, Lee MJ, Barnes JL, Kasinath BS, Feliens D. Acute hyperglycemia rapidly stimulates VEGF mRNA translation in the kidney. Role of angiotensin type 2 receptor (AT2). *Cell Signal.* 2010; 22:1849–1857. [PubMed: 20667471]
- de P Rodrigues SF, dos Santos RA, Silva-Antonialli MM, Scavone C, Nigro D, Carvalho MH, et al. Differential effect of losartan in female and male spontaneously hypertensive rats. *Life Sci.* 2006; 78:2280–2285. [PubMed: 16337658]

- Diniz GP, Carneiro-Ramos MS, Barreto-Chaves ML. Angiotensin type 1 receptor mediates thyroid hormone-induced cardiomyocyte hypertrophy through the Akt/GSK-3 $\beta$ /mTOR signaling pathway. *Basic Res Cardiol*. 2009; 104:653–667. [PubMed: 19588183]
- Griendling KK, Lassegue B, Alexander RW. Angiotensin receptors and their therapeutic implications. *Annu Rev Pharmacol Toxicol*. 1996; 36:281–306. [PubMed: 8725391]
- Hashimoto N, Maeshima Y, Satoh M, Odawara M, Sugiyama H, Kashihara N, et al. Overexpression of angiotensin type 2 receptor ameliorates glomerular injury in a mouse remnant kidney model. *Am J Physiol Renal Physiol*. 2004; 286:F516–F525. [PubMed: 14583437]
- Ideura H, Hiromura K, Hiramatsu N, Shigehara T, Takeuchi S, Tomioka M, Sakairi T, Yamashita S, Maeshima A, Kaneko Y, Kuroiwa T, Kopp JB, Nojima Y. Angiotensin II provokes podocyte injury in murine model of HIV-associated nephropathy. *Am J Physiol Renal Physiol*. 2007; 293:F1214–F1221. [PubMed: 17652372]
- Kimmel PL, Barisoni L, Kopp JB. Pathogenesis and treatment of HIV-associated renal diseases: lessons from clinical and animal studies, molecular pathologic correlations, and genetic investigations. *Ann Intern Med*. 2003; 139:214–226. [PubMed: 12899589]
- Kumar D, Konkimalla S, Yadav A, Sataranatarajan K, Kasinath BS, Chander PN, Singhal PC. HIV-associated nephropathy: role of mammalian target of rapamycin pathway *Am J Pathol*. 2010; 177:813–821.
- Kumar D, Salhan D, Magoon S, Torri DD, Sayeneni S, Sagar A, Bandhlish A, Malhotra A, Chander PN, Singhal PC. Adverse host factors exacerbate occult HIV-associated nephropathy. *Am J Pathol*. 2011; 179:1681–1692. [PubMed: 21871425]
- Kumar D, Plagov A, Yadav I, Torri DD, Sayeneni S, Sagar A, Rai P, Adabala M, Lederman R, Chandel N, Ding G, Malhotra A, Singhal PC. Inhibition of renin activity slows down the progression of HIVAN. *Am J Physiol Renal Physiol*. 2012; 303:F711–F720. [PubMed: 22718888]
- Mavroeiidi V, Petrakis I, Stylianou K, Katsarou T, Giannakakis K, Perakis K, Vardaki E, Stratigis S, Ganotakis E, Papavasiliou S, Daphnis E. Losartan affects glomerular AKT and mTOR phosphorylation in an experimental model of type 1 diabetic nephropathy. *J Histochem Cytochem*. 2013; 61:433–443. [PubMed: 23456824]
- Mezzano SA, Ruiz-Ortega M, Egido J. Angiotensin II and renal fibrosis. *Hypertension*. 2001; 38:635–638. [PubMed: 11566946]
- Ozono R, Wang ZQ, Moore AF, Inagami T, Siragy HM, Carey RM. Expression of the subtype 2 angiotensin (AT<sub>2</sub>) receptor protein in rat kidney. *Hypertension*. 1997; 30:1238–1246. [PubMed: 9369282]
- Rai P, Plagov A, Lan X, Chandel N, Singh T, Lederman R, Ayasolla KR, Mathieson PW, Saleem MA, Husain M, Malhotra A, Chander PN, Singhal PC. mTOR plays a critical role in p53-induced oxidative kidney cell injury in HIVAN. *Am J Physiol Renal Physiol*. 2013; 305:F343–F354. [PubMed: 23678040]
- Rehman S, Husain M, Yadav A, Kasinath BS, Malhotra A, Singhal PC. HIV-1 promotes renal tubular epithelial cell protein synthesis: role of mTOR pathway. *PLoS One*. 2012; 7:e30071. [PubMed: 22253885]
- Ruiz-Ortega M, Esteban V, Suzuki Y, Ruperez M, Mezzano S, Ardiles L, et al. Renal expression of angiotensin type 2 (AT<sub>2</sub>) receptors during kidney damage. *Kidney Int*. 2003; (Suppl):S21–S26.
- Salhan D, Sagar A, Kumar D, Rattanavich R, Rai P, Maheshwari S, Adabala M, Husain M, Ding G, Malhotra A, Chander PN, Singhal PC. HIV-associated nephropathy: role of AT<sub>2</sub>R. *Cell Signal*. 2012a; 24:734–741. [PubMed: 22108089]
- Salhan D, Husain M, Subrati A, Goyal R, Singh T, Rai P, Malhotra A, Singhal PC. HIV-induced kidney cell injury: role of ROS-induced downregulated vitamin D receptor. *Am J Physiol Renal Physiol*. 2012b; 303:F503–F514. [PubMed: 22647636]
- Shanmugam C, Llorens-Cortes C, Clauser E, Corvol P, Gasc JM. Expression of angiotensin II AT<sub>2</sub> receptor mRNA during development of rat kidney and adrenal gland. *Am J Physiol*. 1995; 268:F922–F930. [PubMed: 7771520]
- Tejera N, Gomez-Garre D, Lazaro A, Gallego-Delgado J, Alonso C, Blanco J, et al. Persistent proteinuria up-regulates angiotensin II type 2 receptor and induces apoptosis in proximal tubular cells. *Am J Pathol*. 2004; 164:1817–1826. [PubMed: 15111328]

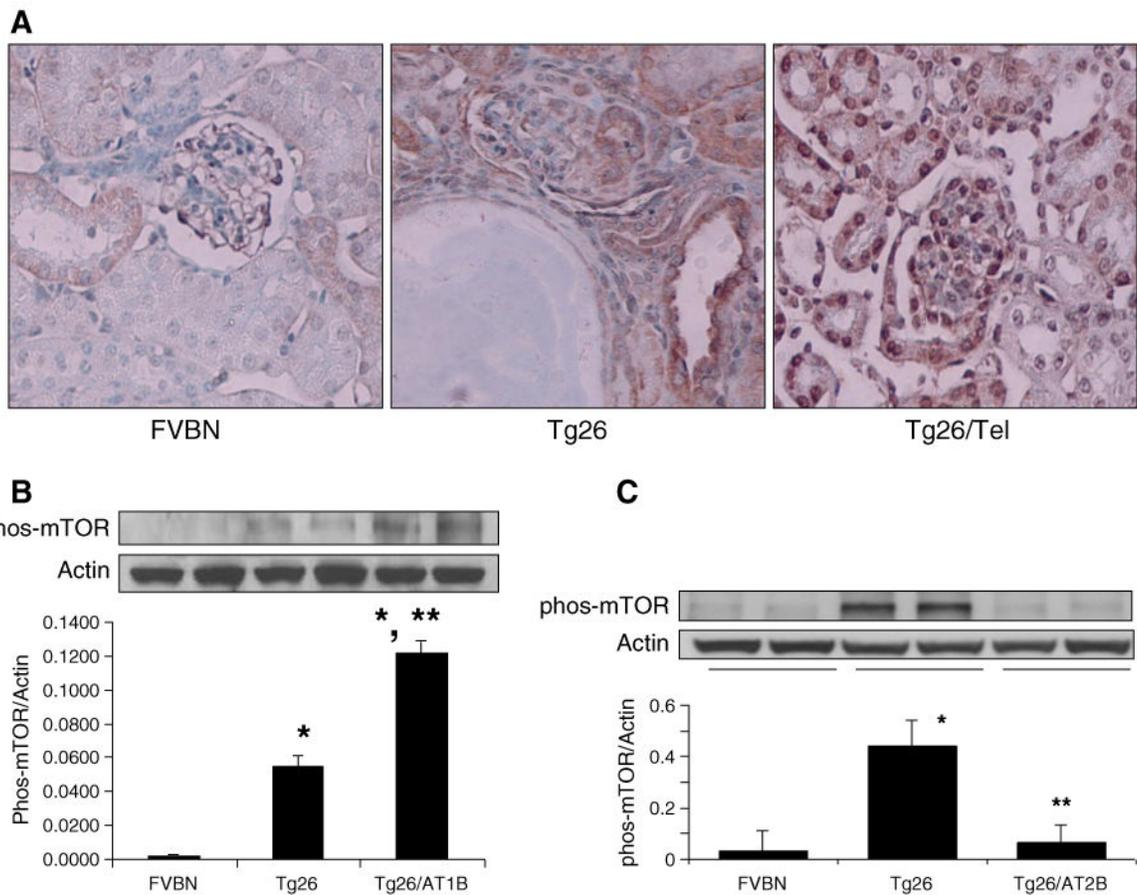
- Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, et al. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev.* 1993; 45:205–251. [PubMed: 8372104]
- Vazquez E, Coronel I, Bautista R, Romo E, Villalon CM, Avila-Casado MC, et al. Angiotensin II-dependent induction of AT(2) receptor expression after renal ablation. *Am J Physiol Renal Physiol.* 2005; 288:F207–F213. [PubMed: 15367388]
- Whaley-Connell A, Habibi J, Panfili Z, Hayden MR, Bagree S, Nistala R, Hyder S, Krueger B, Demarco V, Pulakat L, Ferrario CM, Parrish A, Sowers JR. Angiotensin II activation of mTOR results in tubulointerstitial fibrosis through loss of N-cadherin. *Am J Nephrol.* 2011; 34:115–125. [PubMed: 21720156]
- Wolf G. Renal injury due to renin–angiotensin–aldosterone system activation of the transforming growth factor-beta pathway. *Kidney Int.* 2006; 70:1914–1919. [PubMed: 16985515]
- Yadav A, Vallabu S, Kumar D, Ding G, Charney DN, Chander PN, Singhal PC. HIVAN phenotype: consequence of epithelial mesenchymal transdifferentiation. *Am J Physiol Renal Physiol.* 2010; 298:F734–F744. [PubMed: 20015943]
- Yoshida T, Tabony AM, Galvez S, Mitch WE, Higashi Y, Sukhanov S, Delafontaine P. Molecular mechanisms and signaling pathways of angiotensin II-induced muscle wasting: potential therapeutic targets for cardiac cachexia. *Int J Biochem Cell Biol.* 2013; 45:2322–2332. [PubMed: 23769949]



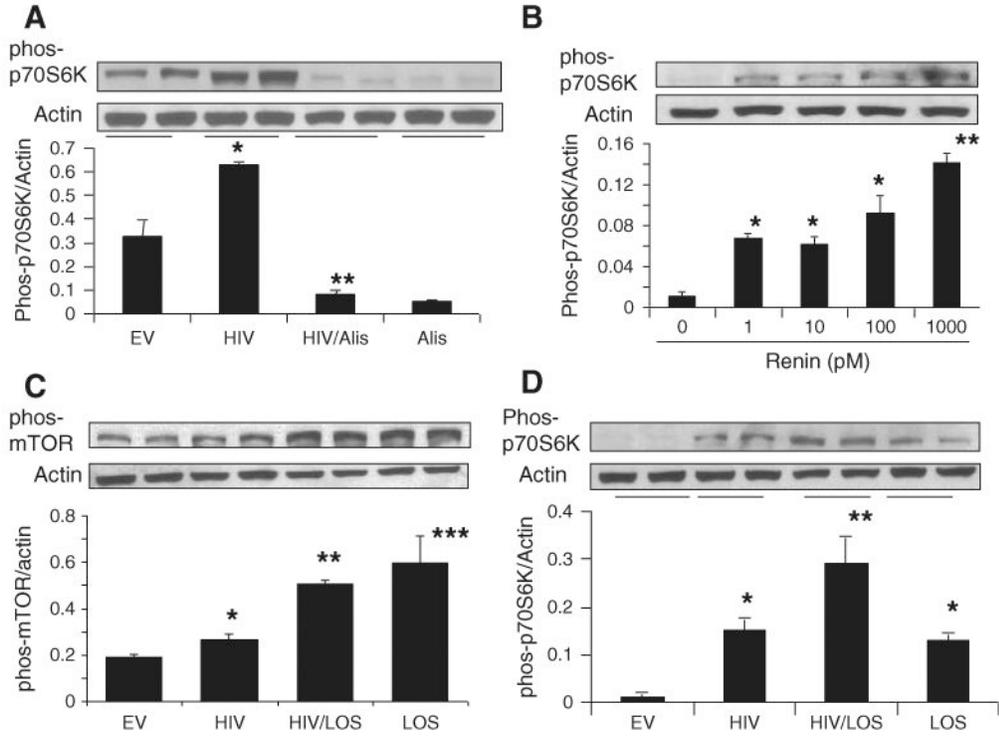
**Fig. 1.** Vpr mice display activation of mTOR pathway in renal tissues. Protein blots of renal tissues of control and Vpr mice were probed for phospho-mTOR and downstream molecules. Representative gels from two mice are shown.



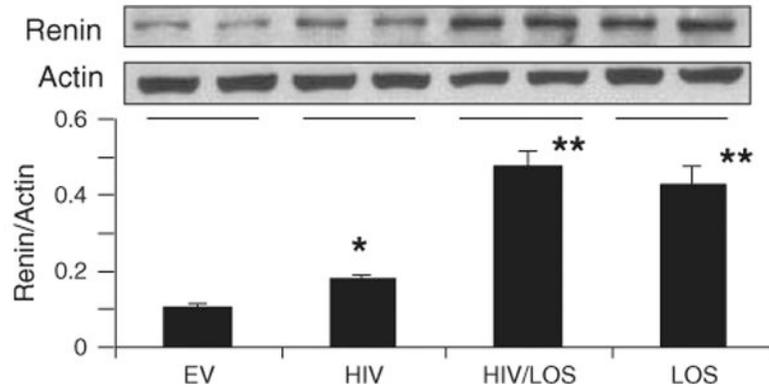
**Fig. 2.** Renin inhibition attenuate renal tissue mTOR phosphorylation and downstream signaling. Protein blots of renal tissues of saline receiving (S1 and S2), Doxy-receiving (D1 and D2), and Doxy/Ali-receiving (D1A1 and D2A2) mice were probed for phospho-mTOR and phospho-p70S6K. The same blots were reprobed for total mTOR and actin. Representative Western blots from lysates from two animals are shown.



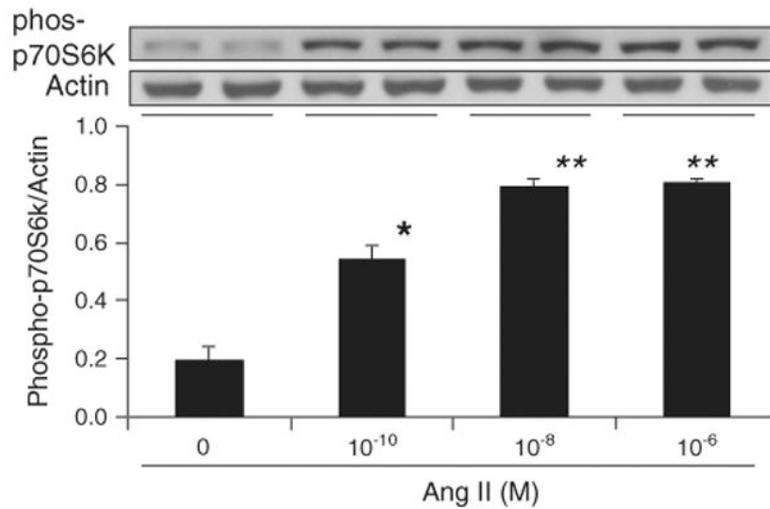
**Fig. 3.** AT1R blockade enhances whereas AT2R blockade attenuated renal tissue mTOR phosphorylation in HIVAN mice. **A.** Renal cortical sections of control, Tg26, and telmisartan receiving Tg26 (Tg26/Tel) mice were immunolabeled for phospho-mTOR. Representative microphotographs are shown. Both podocytes and tubular cells displayed enhanced phosphorylation of mTOR in Tg26 mice. However, kidney cells of Tg26/Tel mice displayed phosphorylation of mTOR predominantly in their nuclei. **B.** Protein blots of renal tissues of control, Tg26, and Tg26/Tel (n = 4) were probed for phospho-mTOR. The same blots were reprobed for actin. Representative gels are shown. Cumulative data for 4 sets of experiments are shown in bar graphs P values. **C.** Protein blots of control, Tg26, and Tg26-receiving AT2B were probed for phospho-mTOR (n = 4). The same blots were reprobed for actin. Representative gels are shown. \*P < 0.01 compared to FVBN; \*\*P < 0.01 compared to Tg26.



**Fig. 4.** Renin or AT1R blockade (losartan) enhance tubular cell mTOR activation. **A.** EV/MTC (EV) and HIV/MTCs (HIV) were incubated in media containing either buffer or aliskiren (Alis,  $10^{-6}$ M) for 24 h ( $n = 3$ ). Protein blots were probed for phospho-p70S6K and the same blots were reprobbed for actin. Representative gels of two different cellular lysates are displayed. Cumulative densitometric data ( $n = 3$ ) data are shown in a bar diagram. \* $P < 0.01$  compared to EV; \*\* $P < 0.001$  compared to HIV alone. **B.** MTCs were incubated in media containing either buffer or variable concentrations of renin (0, 1, 10, 100, and 1000 pM) for 24 h ( $n = 3$ ). Protein blots were prepared and probed for phospho-p70S6K. The same blots were reprobbed for actin. Representative gels are shown. \* $P < 0.01$  compared to renin, 0; \*\* $P < 0.05$  compared to renin, 1–100 pm. **C.** EV/MTC and HIV/MTC were incubated in media containing either buffer or losartan ( $10^{-7}$  M) for 24 h ( $n = 3$ ). Protein blots were probed for phospho-mTOR. The same blots were reprobbed for actin. Representative gels from two different cell lysates are shown. Cumulative data ( $n = 3$ ) are shown in the form of a bar diagram. \* $P < 0.05$  compared to EV; \*\* $P < 0.01$  compared to HIV; \*\*\* $P < 0.01$  compared to EV. **D.** EV/MTC and HIV/MTC were incubated in media containing either buffer or losartan ( $10^{-7}$  M) for 24 h ( $n = 3$ ). Protein blots were probed for phospho-p70S6K. The same blots were reprobbed for actin. Representative gels from two different cell lysates are shown. Cumulative data ( $n = 3$ ) are shown in the form of a bar diagram. \* $P < 0.01$  compared to EV; \*\* $P < 0.05$  compared to HIV alone.

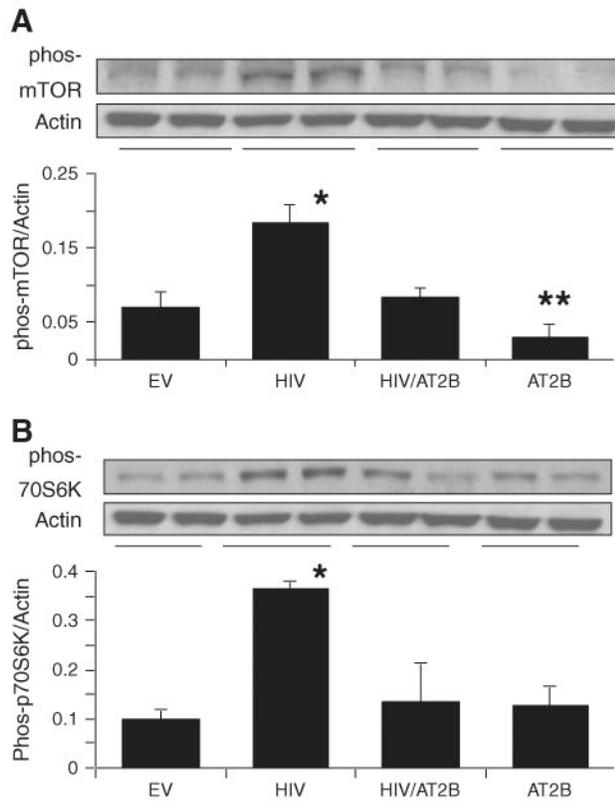


**Fig. 5.** Losartan enhances renin expression under control and HIV milieu. EV/MTCs and HIV/MTCs were incubated in media containing either buffer or losartan ( $10^{-7}$  M) for 24 h. Subsequently, protein blots were probed for renin and reprobbed for actin. Representative gels from two different sets of experiments are shown. Cumulative densitometric data ( $n = 3$ ) are shown in bar graphs. \* $P < 0.05$  compared to EV; \*\* $P < 0.01$  compared to EV and HIV alone.

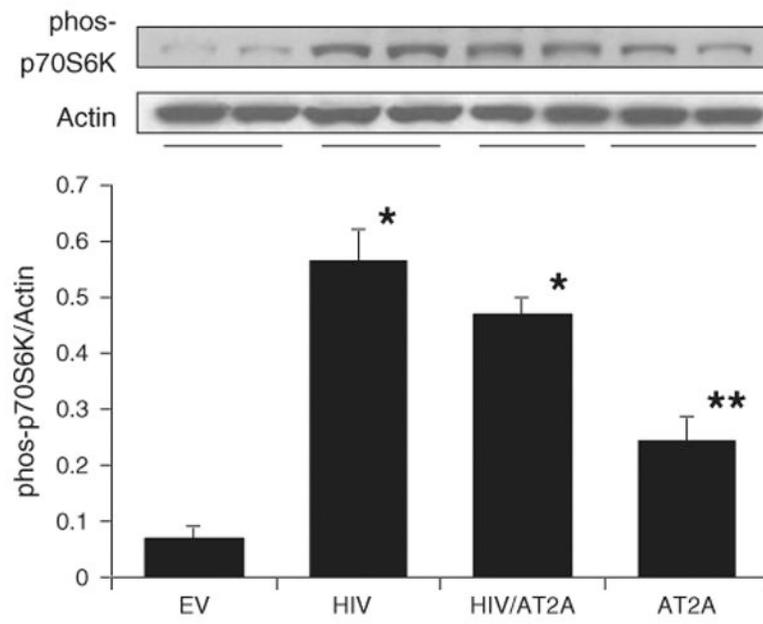


**Fig. 6.**

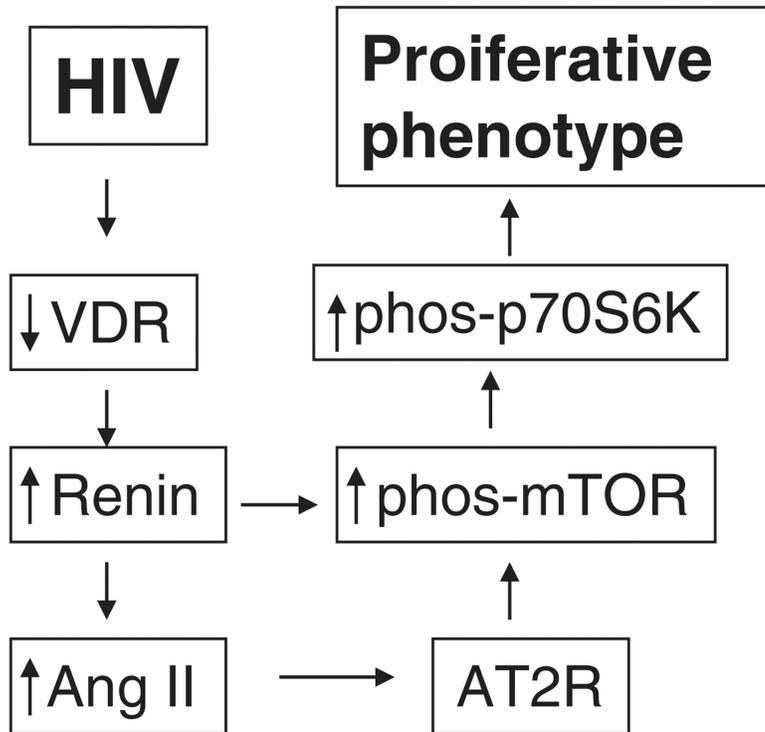
Ang II enhances tubular cell mTOR phosphorylation. EV/MTCs and HIV/MTCs were incubated in media containing variable concentrations of Ang II (0 to 10<sup>-6</sup> M) for 24 h (n = 3). Subsequently, protein blots were probed for phospho-p70S6K. The same blots were reprobbed for actin. Representative gels are shown. Cumulative densitometric data are shown in bar graphs. \*P < 0.01 compared to Ang II, 0; \*\*P < 0.05 compared to Ang II, 10<sup>-8</sup> to 10<sup>-10</sup> M.



**Fig. 7.** AT2R blocker (AT2B) attenuates HIV-induced activation of tubular cell mTOR pathway. A. EV/MTCs and HIV/MTCs were incubated in media containing either buffer or PD123319 (1  $\mu$ M) for 24 h (n = 3). Protein blots were probed for phospho-mTOR and reprobbed for actin. Representative gels are shown. Cumulative data are shown in bar graphs. \*P < 0.01 compared to EV and HIV/AT2B; \*\*P < 0.05 compared to HIV/AT2B. B. EV/MTCs and HIV/MTCs were incubated in media containing either buffer or PD123319 (1  $\mu$ M) for 24 h (n = 3). Protein blots were probed for phospho-p70S6K and reprobbed for actin. Representative gels are shown. Cumulative data are shown in bar graphs. \*P < 0.01 compared to other variables.



**Fig. 8.** AT2 agonist (AT2A) enhance tubular cell phosphorylation of p70S6K. EV/MTCs and HIV/MTCs were incubated in media containing either buffer or AT2A (CGP42112, 1  $\mu$ M) for 24 h (n = 3). Protein blots were probed for phospho-p70S6K and reprobed for actin. Representative gels are shown. \*P < 0.01 compared to EV; \*\*P < 0.05 compared to EV.



**Fig. 9.**  
Proposed schematic diagram.