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Recent advances in tissue (pro)renin imaging

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1. ABSTRACT

Due to its pivotal role in blood pressure control and renal pathologies there is renewed interest in renin and its precursor prorenin. Also, the newly discovered (pro)renin receptor is a new element of the ever broadening renin-angiotensin system (RAS). The complexity of RAS including the recently recognized collecting duct site of (pro)renin (a term denoting both renin and prorenin) synthesis requires the use of advanced research techniques such as multiphoton fluorescence microscopy. With the help of this technology we have pioneered an imaging approach to directly visualize (pro)renin content, release and tissue activity in the living kidney. The use of this technology is reviewed here and exemplified by the direct visualization of (pro)renin activity in the collecting duct. New pharmacological tools, the renin inhibitor aliskiren and the handle region peptide (decoy peptide) was used to further characterize the intra-renal, collecting duct RAS.

2. THE MULTIPHOTON IMAGING APPROACH

Multiphoton excitation laser-scanning fluorescence microscopy is a state-of-the-art imaging approach to study basic organ functions in a real-time, quantitative manner with deep optical sectioning capability and with high spatial and temporal resolution. In contrast to conventional confocal imaging that uses UV or visible laser beams (193-694 nm), multiphoton excitation applies infrared lasers (680-1080 nm) that are advantageous for deep penetration into living tissues with complex, light scattering anatomical structure such as the renal cortex. The technology is based on the concept that the simultaneous absorption of two photons of equal energy can cause excitation of a fluorophore equivalent to the absorption of a single photon with double energy (1-3). With multiphoton imaging, these longer (double) wavelength photons (half of the energy) allow deeper penetration into tissues with much less scattering and phototoxic effects.

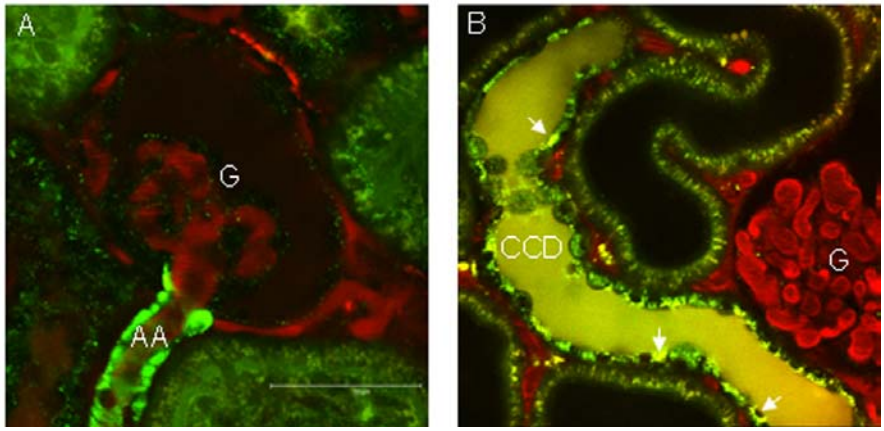

Figure 1. Multiphoton imaging of the living kidney and the intra-renal RAS. *A*: The classic vascular renin site in the juxtaglomerular apparatus. The content of individual renin granules in JG cells of the afferent arteriole (AA) was stained with quinacrine (green). G: glomerulus. The intravascular space (plasma) was labeled using 70 kD dextran-rhodamine (red). *B*: Abundance of quinacrine staining (arrows) in the principal cells of the cortical collecting duct (CCD) from a 12 weeks-old Munich-Wistar-Fromter rat (a model of spontaneous glomerulosclerosis) shows the newly discovered tubular (pro)renin site. Bar is 50 micrometer.

We applied multiphoton microscopy to visualize living renal tissues *in vitro* as well as *in vivo* including direct quantitative imaging of basic functions in renal (patho)physiology in the intact, whole kidney such as single nephron filtration rate (SNGFR) (4), changes in blood flow and tubular flow (4-5), renal concentration, dilution, and permeability of the glomerular filtration barrier (4, 6-7). Very recently we established *in vivo* imaging of cytosolic variables like intracellular pH and $[Ca^{2+}]$ in the intact kidney (3, 8) as well as more integrated and complex functions such as tubuloglomerular feedback (TGF) (4, 9-11). In addition to the above we have successfully visualized renin granule content, release, and tissue renin activity in the juxtaglomerular apparatus (JGA), the main structural component of the renin-angiotensin system (RAS) (12-16) which is described in detail below. In combination with molecular, cell culture and transgenic animal techniques, the multiphoton imaging approach is a novel, complex tool to study the RAS and (pro)renin in health and disease.

3. INTRAVITAL IMAGING OF THE TISSUE RENIN-ANGIOTENSIN SYSTEM (RAS)

The systemic RAS plays a key role in the regulation of blood pressure and in the maintainance of body fluid and electrolyte homeostasis. In addition, in many organs a local tissue RAS is involved in tissue growth, remodeling and development (17). The release of renin from the juxtaglomerular granular cells in the kidney is considered as the rate limiting step of RAS activation and it is controlled by several factors such as the sympathetic nervous system, renal perfusion pressure and the distal tubular salt content at the macula densa. According to the existing paradigm, renin and its biosynthetic precursor prorenin are mainly produced in the kidney by the granular cells of the JGA in the terminal afferent arteriole (18). However, recent work has revealed the expanded presence of the RAS, revealing its complexity compared to the traditional model, in particular in pathological states such as diabetes mellitus. Activation of the intra-renal RAS in diabetes has been well established and it involves dissimilar cell types including mesangial cells, podocytes, immune cells and the tubular epithelium with special regard to the connecting tubule and the cortical collecting duct (CD) as detailed below (19-20).

Renin is known as a hormone, enzyme and more recently a signaling molecule as well (21-23). Considering its pivotal role in RAS activation the renal renin content and release have been studied by using many methods including electron microscopy (24), radioimmunoassays (25), and patch-clamp techniques (26). However, none of these techniques allowed direct visualization of the renin granules in the living tissue and in real-time. Fluorescence imaging of renin granules using the dye quinacrine was first performed in hemorrhagic and ischemic models of rats (27), but the dynamics of renin exocytosis was visualized only recently. Our laboratory established a multiphoton microscopy approach to directly visualize both renin content, release and activity in freshly dissected JGA preparations *in vitro* as well as in the intact kidney *in vivo* with high spatial and temporal resolution down to the individual granule level (3-4, 12-16).

As general rules for any contrast agent, non toxic, water soluble fluorophores must be used with fluorescence imaging applications as well. One good example is quinacrine which is freely permeant to cell membranes and accumulates in the cellular organelles with low pH. This dye clearly and intensely labels renin granules as illustrated in Figure 1A and in previous publications (12-16). However, weak staining can be observed in all cell types most probably due to its accumulation in the lysosomes and cell nuclei as well. Co-localization of intravital quinacrine fluorescence with renin immunofluorescence validated the

use of this dye to label renin granules *in vivo* (3). The application of this imaging approach allowed the investigation of JGA renin secretion in more detail (12-16). Low salt diet for 1 week caused an approximately 5-fold increase in both the number of individual granules and renin-positive JG cells (12). Following treatment with isoproterenol, a beta-agonist, the classic signs of exocytosis were observed by real-time imaging: the association of the emptying granule content with an extracellular cloud of quinacrine fluorescence. The dimming and disappearance of large fluorescence renin granules (1-2 micrometer in diameter) were fast and took place within 300 ms, while no signs of trafficking were observed (12).

In addition to imaging renin content and release, the enzymatic activity of renin or that of activated prorenin can be visualized. A new fluorogenic peptide substrate has been recently developed for renin (AnaSpec, San Jose, CA) which contains a pair of donor-acceptor fluorophores connected by a sequence (decapeptide) of rodent angiotensinogen, including the renin cleavage site at the Leu-Leu bond (a substrate for human renin is available as well). In the absence of renin activity, the donor (5-FAM) fluorescence is quenched by the acceptor molecule (QXL™ 520) due to their close proximity and fluorescence resonance energy transfer (FRET) between them. However, following cleavage into two separate fragments by rat or mouse renin, the intense green fluorescence of 5-FAM is recovered, and can be detected. This fluorescence-based technique permits real-time measurement of renin activity, does not utilize radioactivity, and is conveniently performed within minutes, as opposed to conventional renin assays using radioimmuno-methods. We recently reported that by using this technique the most intense renin activity was observed in the renin-containing terminal part of the afferent arteriole, in the JGA and inside the glomerulus (3, 12). In addition to active renin, this fluorogenic renin substrate can be used to measure the enzymatic activity of activated prorenin (32). The linear relationship between the amount of (pro)renin and the fluorescence intensity of the renin substrate was demonstrated in an earlier study from our laboratory which found 3-35-fold elevations in (pro)renin activity in plasma, renal tissue and cells in response to diabetes and angiotensin II (ANGII) (32). Additional details and examples of imaging renin content, release and activity in the intact kidney tissue can be found in recent reviews on this topic (3, 13-14).

4. (PRO)RENIN IN THE DISTAL NEPHRON

While the JGA is considered to be the classic anatomical site of the RAS within the kidney (18), a body of evidence has revealed that the principal cells of the connecting tubule and collecting duct (CD) can synthesize significant amounts of (pro)renin de novo (28-32). Tubular (pro)renin expression is especially high in pathological states associated with high ANGII such as renovascular hypertension and diabetes mellitus (30-32).

Diabetes mellitus is strongly associated with high RAS activity, high plasma prorenin levels but paradoxically low plasma renin activity (33-34). Using multiphoton imaging we found a significant supply of (pro)renin in the CD under diabetic conditions which finding was confirmed using specific molecular techniques (32). Conversion of prorenin to renin by trypsinization revealed that the vast majority of CD renin is actually prorenin leading to the conclusion that the CD is the major source of prorenin in diabetes (32). Previous studies by our laboratory and others showed that ANGII differentially regulates the synthesis of JGA renin (inhibition) and cortical CD renin (stimulation) (30-32). These findings offered an explanation for the paradoxically high prorenin levels and the persistent RAS activity despite the suppressed JGA in diabetes (32).

What is the functional significance of this local, distal nephron RAS? Interestingly, (pro)renin seems to be secreted toward both the tubular lumen and the interstitium suggested by the high density of (pro)renin granules visualized at both the apical and basal regions of principal cells (32). Cells of the proximal tubule secrete angiotensinogen into the tubular fluid under the same high ANGII conditions, which spills over the entire nephron and collecting ducts (17, 35). Angiotensinogen in the tubular fluid of the collecting duct could be cleaved by the locally secreted (pro)renin (32) with further conversion to ANGII by luminal ACE (36). It is well known that the distal nephron-CD segments contains ANGII type 1 receptors (17, 37). This local, intra-tubular RAS can facilitate salt reabsorption by acting on the epithelial Na^+ channel (38). On the other hand, (pro)renin release into the interstitium can exert both systemic endocrine effects via reabsorption into peritubular capillaries and paracrine effects via the recently described (pro)renin receptor [(P)RR] (39*)*. Taken together, the exact role of the distal nephron-CD RAS is likely complex and it should be further studied. Multiphoton microscopy offers great advantages for future work in this area of research using cortical and medullary tissues *in vitro* or the intact kidney *in vivo* (Figure 1 A, B).

5. EFFECTS OF THE HANDLE REGION PEPTIDE (HRP)

The recently identified (pro)renin receptor [(P)RR] functions to amplify renin enzymatic activity (ANGI generation) and to trigger intracellular signaling (39-40). In addition to renin, the (P)RR binds prorenin equally well and its binding to (P)RR causes a conformational change in prorenin leading to non-proteolytic activation and full enzymatic activity (39-40). (P)RR activation by (pro)renin also results in intracellular signaling via the ERK1/2 MAP kinases, p38 and COX-2 which are essential players in various pathological processes including cell proliferation and tissue fibrosis (39-46).

Previous immunohistochemical studies localized the (P)RR to glomerular mesangial cells, vascular smooth muscle cells of renal and coronary arteries on frozen sections, while it was observed in glomerular mesangial cells and in distal and collecting tubular cells, along their basolateral cell membrane, using paraffin embedded tissue (45).

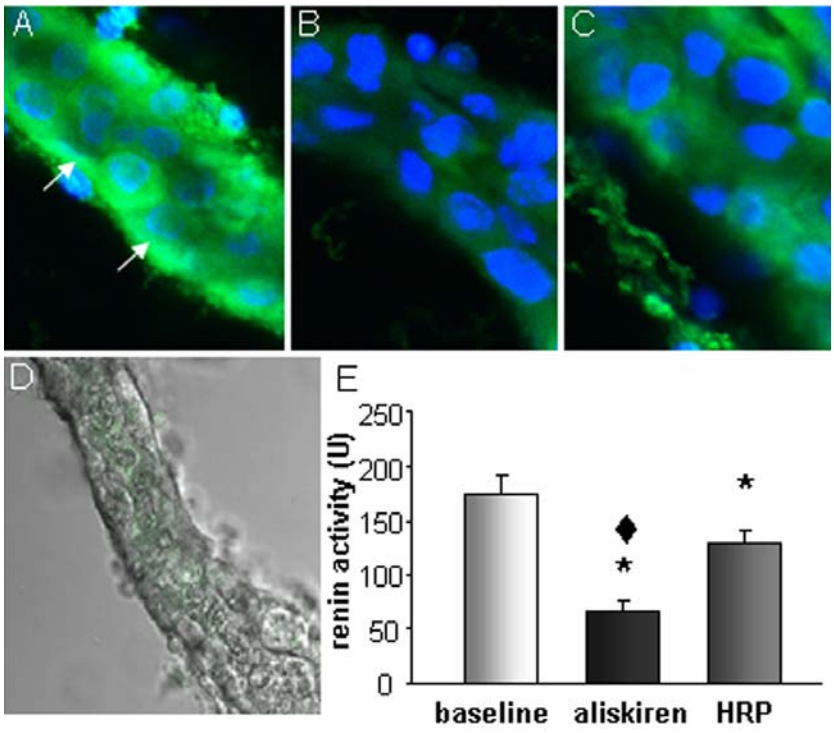

Figure 2. Visualization of (P)RR localization and activity in the living renal collecting duct by fluorescence imaging of the enzymatic activity of (pro)renin. *A-C*: Representative fluorescence images of the *in vitro* CCD preparation incubated with the fluorogenic renin substrate (green) under control conditions (*A*), and after preincubation with 100µM aliskiren (*B*) or 100microM handle region peptide (HRP) (*C*). Nuclei are stained blue with Hoechst33342. Note the high enzymatic activity of (pro)renin in control tissue (*A*) and the pattern of labeling that suggests membrane-bound (pro)renin activity along the basolateral cell membrane and its infoldings (arrows). Both aliskiren (*B)* and HRP *(C)* pretreatment reduced (pro)renin activity in the CCD. *D:* Overlay of the differential interference contrast (DIC) image and the green fluorescence channel shows very low tissue autofluorescence before incubation with the fluorogenic renin substrate. *E:* Summary of (pro)renin activity in the CCD under baseline conditions (n=19), demonstrating the inhibitory effects of aliskiren (n=10) and the (P)RR decoy peptide HRP (n=11). * $p < 0.05$ vs. baseline, ♦ $p < 0.05$ vs. HRP pretreatment. Data are shown as average ± SEM.

To gain further insights in the function of the (P)RR in the kidney we now report for the first time the visualization of CCD (pro)renin activity (either active renin or (P)RR-bound, activated prorenin) using multiphoton fluorescence microscopy. This approach was similar to our previous studies using the FRET-based fluorogenic renin substrate to visualize renin and its activity in the JGA (3, 12). The imaging of tissue (pro)renin activity *in situ* proved to be a great tool to indirectly visualize the localization and activity of the (P)RR in real-time. The isolated CCDs were freshly dissected from C57BL6 mouse kidneys and prepared as described before by our laboratory (47), but were not perfused in the present study. Similarly, the methods of imaging and the use of the fluorogenic renin substrate have been reported earlier in detail (3, 12). The addition of 10 microM renin FRET substrate (5-FAM/QXL™ 520, AnaSpec, San Jose, CA) to the bathing solution resulted in the labeling of most cells of the CCD within seconds (Figure 2A). In addition to significant intracellular labeling, the most intense (pro)renin activity was found at the base of the tubular cells. The pattern of labeling was reminiscent of the basolateral cell membrane and its infoldings (Figure 2A). There was no detectable fluorescence in the bathing solution surrounding the tubule preparation suggesting that the (pro)renin activity (ANGI generation) was membrane-bound. These findings are consistent with the immunohistochemical localization of the (P)RR by Nguyen who found intense labeling at the basolateral membrane of the distal tubule and CCD (45).

Next we applied newly developed pharmacological tools to support (pro)renin and (P)RR-specificity of these data. It is known that the (P)RR interacts with (pro)renin via its handle region which is essential to maintain the prosegment over the active site. Due to its binding to the (P)RR, prorenin undergoes a conformational change and as a result the active site becomes accessible for angiotensinogen (or the renin substrate in our case) as well as for a renin inhibitor. The so-called handle region peptide (HRP) or decoy peptide (NH2-IPLKKMPS-COOH) has been recently developed that mimics a specific amino acid sequence in the prorenin handle region within the prosegment and acts as a peptide antagonist of (P)RR in a competitive manner (46, 48-49). Therefore, in the present experiments we used HRP and aliskiren, a newly developed and marketed renin inhibitor.

Figure 2B shows that the renin substrate's fluorescence intensity ((pro)renin activity) was almost completely prevented by pre-incubating the preparation for 10 min with 100 microM aliskiren. (Pro)renin activity in cells of the CCD was 174±13 (average±SEM) fluorescence units (U) without (n=19), and 67±8 U with aliskiren pretreatment (n=10, $p<0.05$ using ANOVA followed by post-hoc tests (Bonferroni correction) for multiple comparisons). Importantly, a significant inhibitory effect was observed also when the preparation was pre-incubated with 100 microM HRP (129±13 U, n=11, $p<0.05$)(Figure 2C). Similar pretreatment with a control peptide of unrelated amino acid sequence (NH2-MTRLSAE-COOH) had no effect on baseline (pro)renin activity (158±45 U, n=9). These data indicate that the renin substrate's fluorescence intensity was specifically due to (pro)renin activity and suggests that (pro)renin binding to the (P)RR was an important determinant of tissue (pro)renin activity in terms of both intensity and localization. The level of endogenous tissue fluorescence before the application of the renin substrate (Figure 2D) was below the limits of detection which further supports (pro)renin specificity of our technique. Although both aliskiren and HRP, administered in equimolar concentrations (100 microM) significantly inhibited (pro)renin activity in the CCD, aliskiren was more effective (Figure 2E). Our data are in agreement with the basolateral membrane localization of the (P)RR (45), where the HRP can act by inhibiting either the (P)RR-dependent activation of prorenin or the amplification of the enzymatic effect of mature renin.

6. SUMMARY AND CONCLUSION

The combination of multiphoton fluorescence imaging of the living kidney tissue with new fluorescent tools (like the FRET-based fluorogenic renin substrate) and pharmacological inhibitors (aliskiren, HRP) provide novel experimental approaches to study the localization and activity of the (P)RR in the intact kidney. The present imaging studies provided new clues regarding the function of (P)RR in the renal collecting duct which is a newly recognized site of intra-renal (pro)renin synthesis, particularly in high ANGII states. Consistent with the results of previous (P)RR immunolocalization studies showing intense CCD labeling, and with the important role of (P)RR to amplify ANGI generation, high (pro)renin enzymatic activity was found in the basolateral cell membrane of living CCDs. Importantly, the (P)RR decoy peptide HRP effectively reduced (pro)renin activity in this *in vitro* preparation suggesting that HRP does inhibit at least one function of (P)RR, namely the amplification of ANGI generation by receptor-bound (pro)renin. Since the ability of HRP to block (P)RR activation and subsequent intracellular signaling is currently debated (50-51), the present studies provide an alternative mechanism of HRP's action. Inhibition of ANGI generation by HRP likely resulted in reduced intra-renal RAS activation in the disease models in which HRP was found to be protective. However, HRP was reported to prevent the development of pathology also in mice deficient of the AT1 receptor (22). Clearly, more studies are needed to carefully test the effects of this interesting decoy peptide and the binding characteristics of the (P)RR.

7. ACKNOWLEDGEMENTS

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8. REFERENCES

1. W. Denk, J. Strickler, and W.W: Webb. Two-photon laser scanning fluorescence microscopy. *Science* 248, 73-76 (1990)

2. W. R. Zipfel, R. M. Williams, W. W. Webb. Nonlinear magic: multiphoton microscopy in the biosciences. *Nat Biotechnol* 11, 1369-1377 (2003)

3. J. Peti-Peterdi, I. Toma, A. Sipos, S. L. Vargas: Multiphoton imaging of renal regulatory mechanisms. *Physiology (Bethesda)* 24, 88-96 (2009)

4. J. J. Kang, I. Toma, A. Sipos, F. McCulloch, J. Peti-Peterdi: Quantitative imaging of basic functions in renal (patho)physiology. *Am J Physiol Renal Physiol* 291, F495-502 (2006)

5. L. Rosivall, S. Mirzahosseini, I. Toma, A. Sipos, J. Peti-Peterdi: Fluid flow in the juxtaglomerular interstitium visualized *in vivo*. *Am J Physiol Renal Physiol* 291, F1241-1247 (2006)

6. A. H. Salmon, I. Toma, A. Sipos, P. R. Muston, S. J. Harper, D. O. Bates, C. R. Neal, J. Peti-Peterdi: Evidence for restriction of fluid and solute movement across the glomerular capillary wall by the subpodocyte space. *Am J Physiol Renal Physiol* 293, F1777-1786 (2007)

7. J. Peti-Peterdi. Independent two-photon measurements of albumin GSC give low values: *Am J Physiol Renal Physiol* 296, F1255-1257 (2009)

8. A. Sipos, I. Toma, J. J. Kang, L. Rosivall, J. Peti-Peterdi: Advances in renal (patho)physiology using multiphoton microscopy. *Kidney Int* 72, 1188-1191 (2007)

9. J. Peti-Peterdi, S. Morishima, P. D. Bell, Y. Okada: Two-photon excitation fluorescence imaging of the living juxtaglomerular apparatus. *Am J Physiol Renal Physiol* 283, F197-201 (2002)

10. J. Peti-Peterdi: Confocal imaging and function of the juxtaglomerular apparatus. *Curr Opin Nephrol Hypertens* 14, 53-57 (2005)

11. J. Peti-Peterdi: Calcium wave of tubuloglomerular feedback. *Am J Physiol Renal Physiol* 291, F473-480 (2006)

12. J. Peti-Peterdi, A. Fintha, A. L. Fuson, A. Tousson, R. H. Chow: Real-time imaging of renin release *in vitro*. *Am J Physiol Renal Physiol* 287, F329-335 (2004)

13. I. Toma, J. J. Kang, J. Peti-Peterdi: Imaging renin content and release in the living kidney. *Nephron Physiol* 103, 71-74 (2006)

14. J. J. Kang, I. Toma, A. Sipos, F. McCulloch, J. Peti-Peterdi: Imaging the renin-angiotensin system: an important target of anti-hypertensive therapy. *Adv Drug Deliv Rev* 58, 824-833 (2006)

15. I. Toma, J. J. Kang, A. Sipos, S. Vargas, E. Bansal, F. Hanner, E. Meer, J. Peti-Peterdi: Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. *J Clin Invest* 118, 2526-2534 (2008)

16. S. L. Vargas, I. Toma, J. J. Kang, E. J. Meer, J. Peti-Peterdi: Activation of the succinate receptor GPR91 in macula densa cells causes renin release. *J Am Soc Nephrol* 20, 1002-1011 (2009)

17. H. Kobori, M. Nangaku, L. G. Navar, A. Nishiyama: The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev* 59, 251-287 (2007)

18. J. Schnermann, J. P. Briggs: Function of the juxtaglomerular apparatus: control of glomerular hemodynamics and renin secretion. In: R. J. Alpern and S. C. Hebert, eds. The Kidney Physiology and Pathophysiology, Burlington-San Diego-London: Elsevier Academic Press, 589-626 (2008)

19. S. B. Gurley, T. M. Coffman: The renin-angiotensin system and diabetic nephropathy. *Semin Nephrol* 27, 144-152 (2007)

20. J. Peti-Peterdi, J. J. Kang, I. Toma: Activation of the renal renin-angiotensin system in diabetes--new concepts. *Nephrol Dial Transplant* 23, 3047-3049 (2008)

21. Y. Huang, S. Wongamorntham, J. Kasting, D. McQuillan, R. T. Owens, L. Yu, N. A. Noble, W. Border: Renin increases mesangial cell transforming growth factor-beta1 and matrix proteins through receptor-mediated, angiotensin II-independent mechanisms. *Kidney Int* 69, 105-113 (2006)

22. A. Ichihara, F. Suzuki, T. Nakagawa, Y. Kaneshiro, T. Takemitsu, M. Sakoda, A. H. Nabi, A. Nishiyama, T. Sugaya, M. Hayashi, T. Inagami: Prorenin receptor blockade inhibits development of glomerulosclerosis in diabetic angiotensin II type 1a receptor-deficient mice. *J Am Soc Nephrol* 17, 1950-1961 (2006)

23. J. J. Saris, P. A. 't Hoen, I. M. Garrelds, D. H. Dekkers, J. T. den Dunnen, J. M. Lamers, A. H. Jan Danser: Prorenin induces intracellular signaling in cardiomyocytes independently of angiotensin II. *Hypertension* 48, 564-571 (2006)

24. R. Taugner, C. P. Buhrle, R. Nobiling: Ultrastructural changes associated with renin secretion from the juxtaglomerular apparatus of mice. *Cell Tissue Res* 237, 459-472 (1984)

25. J. N. Lorenz, H. Weihprecht, J. Schnermann, O. Skøtt, J. P. Briggs: Renin release from isolated juxtaglomerular apparatus depends on macula densa chloride transport. *Am J Physiol* 260, F486-493 (1991)

26. U. G. Friis, B. L. Jensen, J. K. Aas, O. Skott: Direct demonstration of exocytosis and endocytosis in single mouse juxtaglomerular cells. *Circ Res* 84, 929-936 (1999)

27. M. Alund: Juxtaglomerular cell activity during hemorrhage and ischemia as revealed by quinacrine histofluorescence. *Acta Physiol Scand* 110, 113-121 (1980)

28. A. Rohrwasser, T. Morgan, H. F. Dillon, L. Zhao, C. W. Callaway, E. Hillas, S. Zhang, T. Cheng, T. Inagami, K. Ward, D. A. Terreros, J. M. Lalouel: Elements of a paracrine tubular renin angiotensin system along the entire nephron. *Hypertension* 34, 1265–1274 (1999)

29. A. Rohrwasser, T. Ishigami, B. Gociman, P. Lantelme, T. Morgan, T. Cheng, E. Hillas, S. Zhang, K. Ward, M. Bloch-Faure, P. Meneton, J. M. Lalouel: Renin and kallikrein in connecting tubule of mouse. *Kidney Int* 64, 2155-2162 (2003)

30. M. C. Prieto-Carrasquero, L. M. Harrison-Bernard, H. Kobori, Y. Ozawa, K. S. Hering-Smith, L. L. Hamm, L. G. Navar: Enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. *Hypertension* 44, 223-229 (2004)

31. M. C. Prieto-Carrasquero, F. T. Botros, J. Pagan, H. Kobori, D. M. Seth, D. E. Casarini, L. G. Navar: Collecting duct renin is upregulated in both kidneys of 2-kidney, 1-clip goldblatt hypertensive rats. *Hypertension* 51, 1590-1596 (2008)

32. J. J. Kang, I. Toma, A. Sipos, E. J. Meer, S. L. Vargas, J. Peti-Peterdi: The collecting duct is the major source of prorenin in diabetes. *Hypertension* 51, 1597-1604 (2008)

33. J. A. Luetscher, F. B. Kraemer, D. M. Wilson, H. C. Schwartz, M. Bryer-Ash: Increased plasma inactive renin in diabetes mellitus. A marker of microvascular complications. *N Engl J Med* 312, 1412-1417 (1985)

34. D. A. Price, L. E. Porter, M. Gordon, N. D. L. Fisher, J. M. F. De'Oliveira, L. M. B. Laffel, D. R. Passan, G. H. Williams, N. K. Hollenberg: The Paradox of the Low-Renin State in Diabetic Nephropathy. *J Am Soc Nephrol* 10, 2382-2391 (1999)

35. H. Kobori, L. M. Harrison-Bernard, L. G. Navar: Enhancement of angiotensinogen expression in angiotensin II-dependent hypertension. *Hypertension* 37, 1329-1335 (2001)

36. P. Komlosi, A. L. Fuson, A. Fintha, J. Peti-Peterdi, L. Rosivall, D. G. Warnock, P. D. Bell: Angiotensin I conversion to angiotensin II stimulates cortical collecting duct sodium transport. *Hypertension* 42, 195-199 (2003)

37. L. M. Harrison-Bernard, L. G. Navar, M. M. Ho, G. P. Vinson, S. S. el-Dahr: Immunohistochemical localization of ANG II AT1 receptor in adult rat kidney using a monoclonal antibody. *Am J Physiol* 273, F170-177 (1997)

38. J. Peti-Peterdi, D. G. Warnock, P. D. Bell: Angiotensin II directly stimulates ENaC activity in the cortical collecting duct via AT(1) receptors. *J Am Soc Nephrol* 13, 1131-1135 (2002)

39. G. Nguyen, F. Delarue, C. Burckle, L. Bouzhir, T. Giller, J. D. Sraer: Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest* 109, 1417-1427 (2002)

40. A. H. Danser, J. Deinum: Renin, prorenin and the putative (pro)renin receptor. *Hypertension* 46, 1069-1076 (2005)

41. M. Sakoda, A. Ichihara, Y. Kaneshiro, T. Takemitsu, Y. Nakazato, A. H. Nabi, T. Nakagawa, F. Suzuki, T. Inagami, H. Itoh: (Pro)renin receptor-mediated activation of mitogen-activated protein kinases in human vascular smooth muscle cells. *Hypertens Res* 30, 1139-1146 (2007)

42. Y. Kaneshiro, A. Ichihara, T. Takemitsu, M. Sakoda, F. Suzuki, T. Nakagawa, M. Hayashi, T. Inagami: Increased expression of cyclooxygenase-2 in the renal cortex of human prorenin receptor gene-transgenic rats. *Kidney Int* 70, 641-646 (2006)

43. Y. Huang, N. A. Noble, J. Zhang, C. Xu, W. A. Border: Renin-stimulated TGF-beta1 expression is regulated by a mitogen-activated protein kinase in mesangial cells. *Kidney Int* 72, 45-52 (2007)

44. A. Ichihara, Y. Kaneshiro, T. Takemitsu, M. Sakoda, T. Nakagawa, A. Nishiyama, H. Kawachi, F. Shimizu, T. Inagami: Contribution of nonproteolytically activated prorenin in glomeruli to hypertensive renal damage. *J Am Soc Nephrol* 17, 2495-2503 (2006)

45. G. Nguyen: Increased cyclooxygenase-2, hyperfiltration, glomerulosclerosis, and diabetic nephropathy: put the blame on the (pro)renin receptor? *Kidney Int* 70, 618-620 (2006)

46. S. Satofuka, A. Ichihara, N. Nagai, T. Koto, H. Shinoda, K. Noda, Y. Ozawa, M. Inoue, K. Tsubota, H. Itoh, Y. Oike, S. Ishida: Role of nonproteolytically activated prorenin in pathologic, but not physiologic, retinal neovascularization. *Invest Ophthalmol Vis Sci* 48, 422-429 (2007)

47. A. Sipos, S. L. Vargas, I. Toma, F. Hanner, K. Willecke, J. Peti-Peterdi: Connexin 30 deficiency impairs renal tubular ATP release and pressure natriuresis. *J Am Soc Nephrol* 20, 1724-1732 (2009)

48. A. Ichihara, M. Hayashi, Y. Kaneshiro, F. Suzuki, T. Nakagawa, Y. Tada, Y. Koura, A. Nishiyama, H. Okada, M. N. Uddin, A. H. Nabi, Y. Ishida, T. Inagami, T. Saruta: Inhibition of diabetic nephropathy by a decoy peptide corresponding to the "handle" region for nonproteolytic activation of prorenin. *J Clin Invest* 114, 1128-1135 (2004)

49. M. N. Uddin, A. H. Nabi, T. Nakagawa, A. Ichihara, T. Inagami, F. Suzuki F: Non-proteolytic activation of prorenin: activation by (pro)renin receptor and its inhibition by a prorenin prosegment, "decoy peptide". *Front Biosci* 13, 745-753 (2008)

50. S. Feldt, U. Maschke, R. Dechend, F. C. Luft, D. N. Muller: The putative (pro)renin receptor blocker HRP fails to prevent (pro)renin signaling. *J Am Soc Nephrol* 19, 743-748 (2008)

51. S. Feldt, W. W. Batenburg, I. Mazak, U. Maschke, M.Wellner, H. Kvakan, R. Dechend, A. Fiebeler, C. Burckle, A. Contrepas, A. H. Jan Danser, M. Bader: Prorenin and renin-induced extracellular signal-regulated kinase 1/2 activation in monocytes is not blocked by aliskiren or the handle-region peptide. *Hypertension* 51, 682-688 (2008)

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