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Biochemical, Structural and Pathophysiological Aspects of Prorenin and (Pro)renin Receptor

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1. Introduction

Our knowledge of understanding the complex role of renin angiotensin system (RAS) or RA system in human physiology has been widened for more than 100 years and it is rapidly increasing day-by-day. Over the last two decades discoveries of angiotensin converting enzyme 2 (ACE 2), putative receptor for angiotensin (Allen et al., 2006; Aronsson et al. 1988; Bickerton & Buckley, 1961; Cooper et al., 1996; Deschepper et al., 1986; Dzau et al., 1986; Epstein et al., 1970), and (pro)renin receptor have laid the foundation of many new hypotheses in the context of their biochemical actions, physiological effects and activation of second messenger pathways. Thus, scientists have started to reconsider the complex biochemistry and physiology of RAS. The primary and main role of this system is to regulate homeostasis of body fluid that ultimately maintains the blood pressure (Kobori et al., 2006; Oparil & Haber, 1974a; Oparil & Haber; 1974b). This system catalyzes a liver product, angiotensinogen, to generate a small decapeptide, angiotensin-I (Ang-I). Angiotensin converting enzyme (ACE), thus, converts Ang-I into octapeptide, angiotensin-II (Ang-II). Ang-II acts directly within the central nervous system to increase blood pressure (Bickerton & Buckley, 1961). Injection of purified Ang-II peptide around the hypothalamus in rat brain stimulated thriving drinking response (Epstein et al., 1970). The physiological actions of the most potent hormone peptide are mediated via G-protein coupled angiotensin II type 1 (AT1) and angiotensin II type 2 (AT2) receptors. Ang-II facilitates vasoconstriction, cell proliferation, cell hypertrophy, anti-natriuresis, fibrosis, atherosclerosis using AT1 (Ito et al., 1995) while, via AT2 receptor, the peptide elicits vasodilation, anti-proliferation, anti-hypertrophy, anti-fibrosis, anti-thrombosis, anti-angiogenesis (Siragy & Carey, 1997; Goto et al., 1997; Gross et al., 2000). The classical renin angiotensin system with the generation of different peptides and their physiological effects has been presented in Figure 1.

The systemic or classical renin angiotensin system has usually been viewed as the blood-borne cascade whose ultimate product Ang-II plays the pivotal endocrine role. Plasma renin activity is the most accepted clinical marker of circulating RAS. However, circulating RAS remained unsuccessful to describe the autocrine and paracrine functions mediated by RAS within specific tissue sites particularly in heart, kidney, brain and vasculature. Transgenic

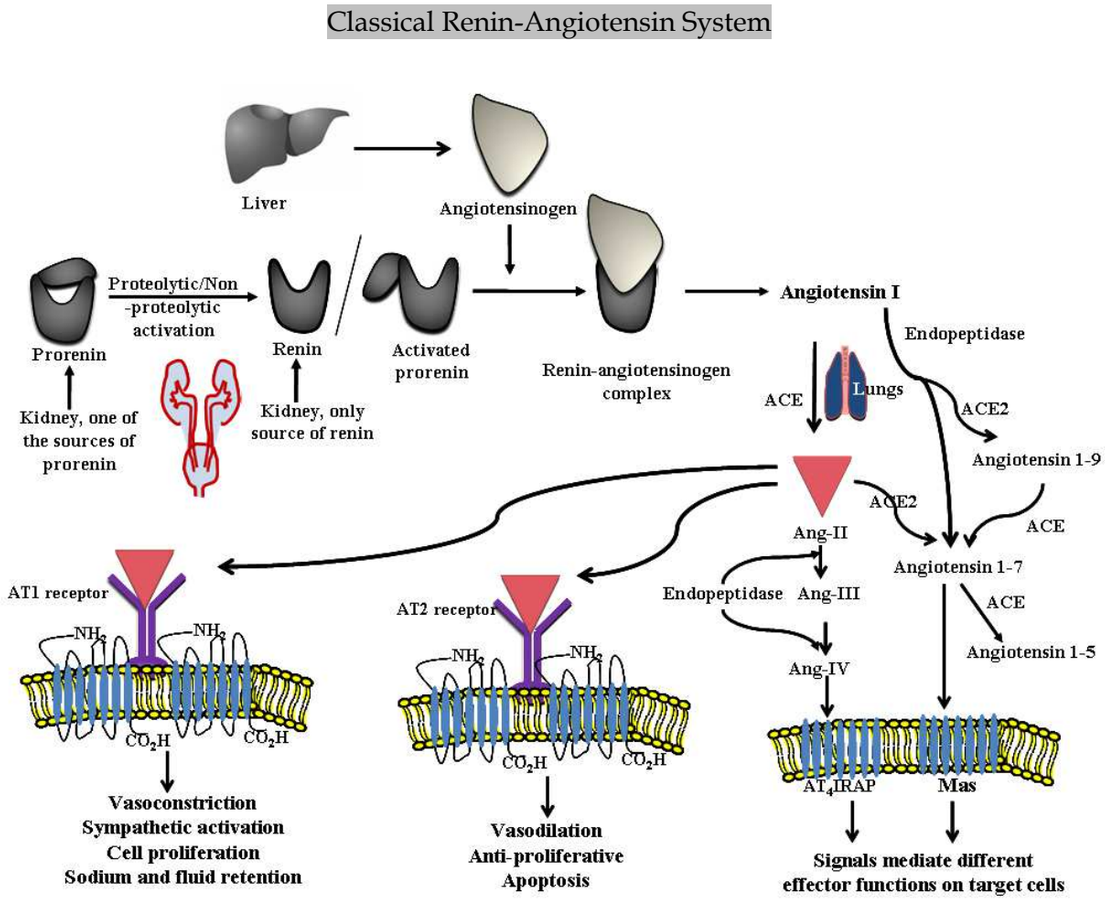

Fig. 1. Schematic diagram of the classical renin-angiotensin (RA) system shows angiotensin-II dependent pathway mediated different physiological effects via angiotensin type 1 (AT1) and type 2 (AT2) receptors. Renin, secreted from kidney, regulates the rate limiting step of this pathway by converting its liver originated macromolecule substrate angiotensinogen into a short peptide, angiotensin-I (Ang-I). Angiotensin-I, thus, is converted into angiotensin-II (Ang-II) by angiotensin converting enzyme. Other peptide products via stimulation of enzymes and receptor subtypes on target cells can also mediate physiological functions.

animals facilitate to demonstrate the existence of tissue RAS parallel to and independent of systemic RAS. Thus, local RA system has been ensured in intracellular compartments (de Mello, 1995; van Kesteren et al., 1997; Admiraal et al., 1999), interstitial fluids (de Lannoy et al., 1997, 1998), cardiac cells including fibroblasts, endothelial cells, myocytes, macrophages (de Lannoy et al., 1998; Hokimoto et al., 1996; Sun et al., 1994) as well as on the cell membrane (Danser et al., 1992; Neri Serneri et al., 1996). All the circulating components of renin angiotensin system i.e., renin, angiotensinogen, ACE, and Ang I and II though not produced but have been identified in cardiac tissue (Campbell et al., 1993; Danser et al., 1994). As a consequence, presence of local RAS in the heart could contribute to the pathogenesis of congestive heart failure, cardiac hypertrophy and remodeling, and reperfusion arrhythmias (Yusuf et al., 1991: Ruzicka et al., 1994; Schieffer et al., 1994; Van

Gilst et al., 1984). Direct action of Ang II within the central nervous system causes increased blood pressure. Also, presence of renin and endogenous production of angiotensin have established the existence of local RAS in the central nervous system (Bickerton and Buckley et al., 1961; Fischer-Ferraro et al., 1971; Ganten et al., 1971).

1.1 The main players associated with renin angiotensin system

The RA system is initiated by its rate-limiting enzyme renin (37 kDa with 340 amino acid residues) which catalyzes its only known substrate angiotensinogen. Renin is only secreted from kidney as preprorenin and levels of renin in the plasma of nephrectomized animals is not detectable. Professor Robert A. Tigerstedt and his student Per G. Bergman for the first time reported a "pressor" substance in the kidney extract more than 100 years ago, which caused increase in blood pressure in experimental animals and later, coined that substance as renin (Tigerstedt & Bergman, 1898). Renin, also known as aspartyl proteinase having an optimum pH of 5.5 to 7.5 instead of 2.0 to 3.4, has no known physiological effect other than the proteolysis of angiotensinogen (Murakami & Inagami, 1975; Inagami & Murakami, 1977; Matoba *et al*., 1978; Figueiredo *et al*., 1985: Dzau *et al*. 1979; Yokosawa *et al*., 1978; Yokosawa, 1980; Hirose, 1982; Pickens *et al*., 1965). The neutral pH is necessary to show its activity in plasma. The renin gene is also expressed in other tissues such as adrenal gland, gonads, placenta, pituitary, brain and hypothalamus (Hirose et al., 1978; Naruse et al., 1981, 1982; Pandey et al., 1984; Deschepper et al., 1986; Dzau et al., 1987; Paul et al., 1987; Suzuki et al., 1987; Tada et al., 1989). These extra renal renins have been thought to play a part in the tissue renin-angiotensin system proposed by several investigators (de Mello, 1995; van Kesteren et al., 1997; Admiraal et al., 1999; de Lannoy et al., 1997, 1998; Hokimoto et al., 1996; Sun et al., 1994; Danser et al., 1992, 1994; Neri Serneri et al., 1996; Campbell et al., 1993; Yusuf et al., 1991: Ruzicka et al., 1994; Schieffer et al., 1994; Van Gilst et al., 1984; Bickerton and Buckley et al., 1961; Fischer-Ferraro et al., 1971; Ganten et al., 1971).

Removal of the 23 amino acid residues from the C-terminus of preprorenin generates prorenin. Prorenin (45-47 kDa containing 406 amino acid residues), the pre-active form of renin, is predominantly synthesized by granular cells of the juxtaglomerular apparatus (JGA) in the terminal afferent arteriole (Schnermann & Briggs, 2008; Schweda et al., 2007) and principle cells of the collecting ducts (Prieto-Carrasquero et al., 2004; Rohrwasser et al., 1999; Kang et al., 2008). Prorenin is also synthesized in many other tissues like adrenal glands (Ganten et al., 1974, 1976; Ho and Vinson, 1998), zona glomerulosa (Doi et al., 1984; Deschepper, et al., 1986; Brecher et al., 1989), eye, Müller cells, mast cells (Krop et al., 2008), ovarian follicular fluid (Glorioso et al., 1986), and theca cells (Do et al., 1988), uterus (Derkx et al., 1987; Itskovitz et al., 1987), myometrium/decidual cells (Shaw et al., 1989), placenta (Lenz et al., 1991), chorionic cells, testis and leydig cells (Sealey et al., 1988). The submandibular gland in some mice strains produces a large amount of renin, which is a product of the Ren-2 renin gene distinct from the renal renin gene, Ren-1 (Cohen et al., 1972; Wilson et al., 1981; Holm et al., 1984) and this action is mediated by prorenin converting enzyme present in submandibular gland of the same mice strains (Kim et al., 1991). Prorenin, in the juxtaglomerular cells of the kidney, is converted to mature renin by the limited endoproteolysis after paired basic residues, Lys-Arg to remove the 43-amino acid residues containing prosegment sequence. The concentration of prorenin in human plasma

is 10 times higher than that of mature renin though the physiological role of prorenin is still not clear and the relative concentration of prorenin to renin varies at different conditions. Thus, conversion of prorenin i.e., activation of prorenin to renin plays important role in the regulation of RA system. Certain proteases like trypsin or cathepsin were found to activate prorenin by cleaving the residue prosegment reversibly (Inagami et al., 1980; Shinagawa et al., 1990, 1994; Kikkawa et al., 1998; Jutras et al., 1999; Taugner et al., 1985; Wang et al., 1991; Jones et al., 1997). However, many tissues store prorenin but do not process it to active renin. Though extra-renal sources of prorenin are evident, kidney is the major source of plasma prorenin. Renin and prorenin have long been considered as the separate mediators of tissue and circulating systems (Sealy & Rubattu, 1989). *In vitro*, when prorenin is acidified at pH 3.3 or exposed to low temperature (< 4˚C) or allowed to interact with antibodies designed from its prosegment sequences (protein-protein interaction), it mediates intrinsic catalytic activity without removal of the prosegment sequence from its N-terminus through a reversible change in conformation (Derkx et al., 1979, 1983, 1987a & b, 1992; Pitarresi et al., 1992, Suzuki et al., 2000, 2003).

Both renin and non-proteolytically activated prorenin catalyze angiotensinogen, a 6 kDa protein macromolecule found also in adipose tissues to generate a small decapeptide called angiotensin I. Both neonatal and adult rat cardiac cells express mRNA for angiotensinogen (Dostal et al., 1992; Malhotra et al., 1994; Zhang et al., 1995; Liang et al., 1998; Sadoshima et al., 1993), while van Kesteren and colleagues (1999) were unable to detect angiotensinogen in neonatal rat cardiac cells or in the conditioned medium of these cells using radioimmunoassay. Secreted angiotensinogen in the cultured medium of neuronal cells has been identified. Generated renin product, angiotensin I thus, further converted into angiotensin II by the action of ACE.

The (pro)renin receptor or (P)RR is now considering as another important regulatory component in renin-angiotensin system. However, ongoing research works have revealed its association both in angiotensin II-dependent and –independent pathways which also play pivotal role in the developmental processes.

2. (Pro)renin receptor, a new family member of renin angiotensin system

It's been almost a decade since the full length (pro)renin receptor or (P)RR was cloned (Nguyen et al., 2002). However, earlier the same group (Nguyen et al., 1996, 1998) reported high affinity binding of ^{125}I renin to primary and immortalized human mesangial cells (0.2 and 1.0 nM, respectively) in a time-dependent fashion that could attain saturable state. The (P)RR does not internalize the ligands inside the cells rather activates renin and prorenin after binding to generate angiotensin I and second messenger pathway by activating proteins involved in signaling. In the late nineties of the last century, the mannose 6-phosphate/insulin-like growth factor II (M6P/IGF2) receptor was found on rat cardiac myocytes (van Kesteren et al., 1997) and human endothelial cells (Admiraal et el., 1999; Saris et al., 2001) that could bind and internalize renin/prorenin (van Kesteren et al., 1997). However, such binding and internalization could not generate any angiotensin peptides intracellularly. Besides, existence of renin/prorenin receptor independent of mannose 6-phosphate such as renin binding protein (RnBP), renin/prorenin binding protein (ProBP) in rat tissues, vascular renin binding protein have also been confirmed (Takahashi et al., 1983;

Tada et al., 1992; Campbell et al., 1994; Sealy et al., 1996) that bind with different binding affinities to their ligands.

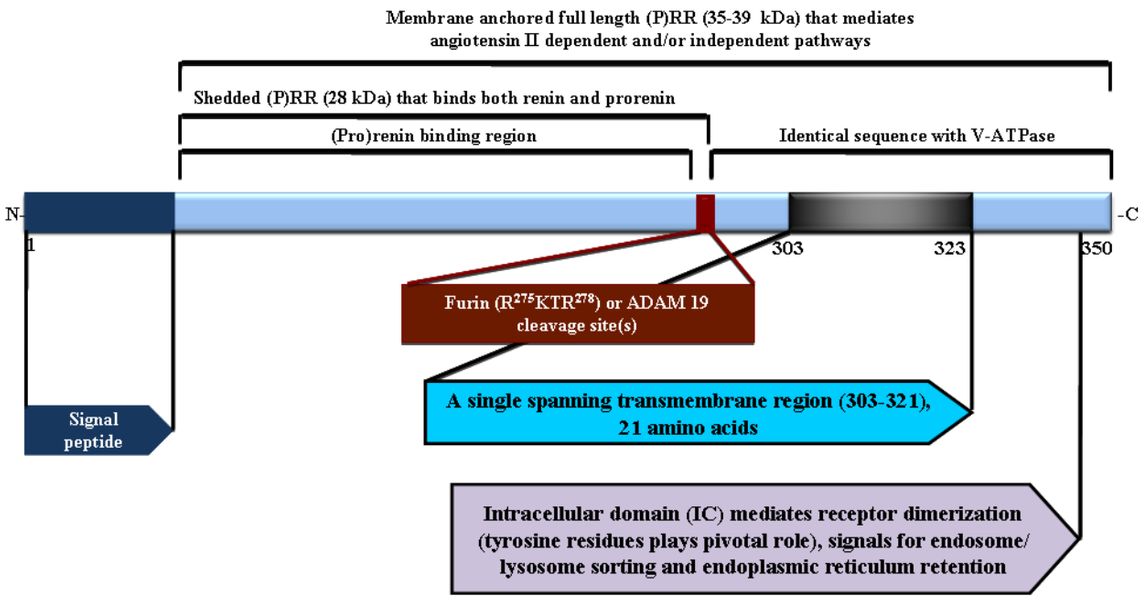

Fig. 2. Structure of (pro)renin receptor protein. The receptor protein is composed of three basic constituents with an N-terminal domain, which is the (pro)renin binding site, a single spanning transmembrane sequence that traverse through the plasma membrane and the intracellular cytoplasmic domain that recently has been identified as the important region required for the dimerization of (P)RR.

The (P)RR, expressed on the cell surface, is a 350 amino acid (39 kDa) containing protein with a single spanning transmembrane domain encoded from the X-chromosome. A short signal peptide is present at the N-terminus end of the unglycosylated large extracellular domain with ~310 residues and the transmembrane domain has putative 20-amino acid residues followed by a ~19-amino acid containing intracellular cytoplasmic (IC) domain (shown in Figure 2). Ubiquitous expression of (P)RR has been demonstrated with the highest amount of mRNA found in brain, heart and placenta while lower amount was expressed in liver, pancreas and kidney (Nguyen et al., 2002). It is reported that (P)RR expressed in VSMCs in human (P)RR transgenic rats can be recycled between intracellular compartment and cell membrane (Batenburg et al., 2007). The (P)RR is also localized on the membrane of stromal adipose cells (Achard et al., 2007), in the neurons of neonatal rats (Shan et al., 2008), on COS-7 cells (Nabi et al., 2007), in glomerular mesangial cells, the subendothelium of renal arteries, podocytes, and distal nephron cells (Nguyen et al., 2002, 1996, 1998) of human and rat kidneys; U937 monocytes (Feldt et al., 2008b) and also in intracellular compartments or on the surface of vascular smooth muscle cells (VSMCs) (Sakoda et al., 2007; Zhang et al, 2008), in endoplasmic reticulum (Schefe et al., 2006; Yoshikawa et al., 2011), golgi apparatus (Contrepas et al., 2009; Yoshikawa et al., 2011), cytosol (Contrepas et al., 2009; Cousin et al., 2009) and found in plasma (Cousin et al., 2009). Expression of (P)RR is also categorized in the subfornical organ (SFO), paraventricular nucleus, the supraoptic nucleus, the nucleus of the tractus solitarius (NTS), or the rostral

ventrolateral medulla regions of brain that were believed to be involved in the central regulation of cardiovascular function and volume homeostasis (Contrepas et al., 2009), in the frontal lobe of human brain and pituitary (Takahashi et al., 2010). Retina is also a source of (P)RR (Satofuka et al., 2009). In particular, it is localized to pericytes in retinal vessels, endothelial cells, and, mostly in retinal ganglion cells and glia (Wilkison-Berka et al., 2010). Moreover, predominant expression of (P)RR using immunohistochemistry and *in situ* hybridization on the epical membrane of acid secreting cells in the collecting duct has been reported (Advani et al., 2009).

Full length rat and human recombinant (P)RR with transmembrane followed by cytoplasmic domains were expressed in baculovirus expression system, and identified in the cellular fraction (Nabi et al., 2006; Du et al., 2008; Kato et al., 2008). On the other hand, human (P)RR containing only the extracellular domain lacking transmembrane part was found secreted in the culture medium (Kato et al., 2009). Also, human (P)RR was successfully expressed in the Bombyx mori multiple nucleopolyhedrovirus (BmMNPV) and found in the silkworm larvae as well as in the fat body of silkworm larvae. ELISA and surface plasmon resonance technique in BIAcore assay system confirmed the renin/prorenin binding ability i.e., the functional bioactivity of (P)RR expressed and fractionated from silkworm and baculovirus expression system (Nabi et al., 2006; Du et al., 2008; Kato et al., 2008, 2009).

A protease called furin was found to be responsible for shedding of endogenous (P)RR in trans-golgi by cutting at positions $R^{275}KTR^{278}$ near the N-terminus of transmembrane sequence (Cousins et al., 2009). This soluble form of (P)RR [s(P)RR, 28 kDa] was detected in the conditioned cultured medium and also in human plasma using co-precipitation experiment with human renin. Another protease ADAM19 sheds intracellular (P)RR from golgi apparatus into the extracellular space (Yoshikawa et al., 2011). Moreover, constitutively secreted soluble form of (P)RR (~30 kDa) shedded from the cell surface was found in the cultured medium of human umbilical vein endothelial cells (HUVECs) (Biswas et al., 2011) that could also bind recombinant human prorenin with a nanomolar order, similar to what was reported for full length (P)RR on the cell surface (Nguyen et al., 2002; Batenburg et al., 2007; Nurun et al., 2007) or from the baculovirus expression system (Nabi et al., 2006). Non-proteolytic activation of prorenin occurred when it interacted with s(P)RR in the soluble phase and this was confirmed by Western blot analysis. Also, activated prorenin showed renin activity by generating Ang I from sheep angiotensinogen (Biswas et al., 2011). However, the enzymatic properties of renin after binding to (P)RR is yet to be determined. These phenomenons have been depicted in Figure 3 (vi).

C-terminal domain of (P)RR is identical to "M8-9," a truncated protein of 8.9 kDa that co-purified with a proton-ATPase of bovine chromaffin granule membranes (Ludwig et al., 1998). At the gene level (P)RR from human, rat, and mice showed 95% sequence homology, while at the amino acid level they showed 80% homology. Phylogenetic analyses also revealed that the sequences for (P)RR are not only conserved within the closely related species but also similar sequences are present in the remote species. The IC-domain of (P)RR mediates the signal transduction pathways and promyelocytic zinc finger (PLZF) protein has been identified as an associated protein that interacts with the IC-domain to down regulate expression of the receptor. (P)RR has also been reported to exist as a dimer *in vivo* (Schefe et al., 2006). Recent evidences suggest that short and relatively flexible loop of IC

segment generates the driving force in the process of dimerization of (P)RR and tyrosine residues of IC contribute in dimerization dominantly (Zhang et al., 2011).

2.1 (Pro)renin receptor and its ligand: interaction of (pro)renin with (pro)renin receptor

Interaction of renin and prorenin with (pro)renin receptor instigates two pathways: one leads to generation of angiotensin II that ultimately contribute to the activation of local RA system via angiotensin II-dependent pathway as in case of classical circulating RA system and the other one leads to signal transduction mediated by angiotensin II-independent pathway outlined in Figure 3.

2.1.1 Binding mechanism and activation of renin angiotensin system

Binding of human renin to human (P)RR increases local angiotensin production as it is manifested by the increased (4/5-fold) substrate affinity of (P)RR-bound renin compare to free form of soluble renin (Nguyen et al., 2002). On the other hand, human renin bound to recombinant human/rat (P)RR and free form of soluble renin showed similar binding affinity for the substrate, sheep angiotensinogen at the micromolar order (Nabi et al, 2006; Nurun et al., 2007). However, kinetic data analyses revealed that prorenin preferentially binds to (P)RR and such binding initiates angiotensin I generation (Nabi et al., 2006; 2009b). Full length rat (P)RR expressed and isolated from the baculovirus expression system had almost 3 times higher binding affinity (K_D = 8.0 nM) for rat prorenin than that of mature rat renin (K_D = 20.0 nM) *in vitro* (Nabi et al., 2006). Receptor-bound rat prorenin also had similar affinity for the substrate (K_m = 3.3 M) sheep angiotensinogen as it was for rat renin. On the other hand, receptor-bound renin showed higher molecular activity (10 nM·h) compared to free form of mature renin and receptor-bound activated prorenin (1.25 and 1.1 nM·h) (Nabi et al., 2006).

Ninety% of rat and fifty% of human prorenin (at 2.0 nM of initial concentration) bound to their respective (P)RR over expressed on the membrane of COS-7 cells and the K_Ds were estimated to be 0.89 and 1.8 nM, respectively. Receptor-bound rat and human prorenin showed 30% and 40% activity, respectively, in comparison with the activity of trypsinized prorenin molecules (Nurun et al., 2007). A similar binding and activation patterns of prorenin to human (pro)renin receptor expressed in VSMCs of transgenic rats (K_D = 6.0 nM) (Baternburg et al., 2007) and of rat prorenin by rat (P)RR expressed in cultured VSMCs were observed (Zhang et al., 2010). Differences in the K_D values of rat prorenin bound to the immobilized receptors on the synthetic surfaces and the membrane-anchored receptor could be due to the presence of some other associated proteins that might have stabilized the (P)RR on the membrane. Surface plasmon resonance technique in BIAcore assay system revealed almost four times higher binding affinity of human prorenin (1.2 nM) to the *in vitro* synthesized human recombinant (P)RR compared to that of human mature renin (4.4 nM) (Nabi et al., 2009b). The immobilized receptors bind recombinant human renin and prorenin with the dissociation constant (K_D) values of 1.2 and 4.4 nM, respectively. Also, the data obtained from the BIAcore kinetic study showed that association rate of prorenin to (P)RR is higher than that of mature renin (1.8×10^7 and 2.16×10^6 $M^{-1}.s^{-1}$, respectively) (Nabi et al., 2009b).

The binding mechanism of renin and prorenin to the (pro)renin receptor has also been proposed depending on the ground work led by Suzuki and colleagues who demonstrated the importance of "handle" ($I^{11P}FLKR^{15P}$) and "gate" ($T^{7P}FKR^{10P}$) region peptides designed from the prosegment sequence of prorenin in the non-proteolytic activation of prorenin via protein-protein interaction (Suzuki et al., 2003). Later, another peptide called "decoy" ($R^{10P}IFLKRMPSI^{19P}$ including the "handle" sequence) that mimics the N-terminus sequence of human prorenin prosegment showed its high binding affinity to the recombinant (P)RR and this affinity explains the probable reason for high binding affinity of prorenin for (P)RR. Decoy peptide has got binding affinity to (P)RR at the nanomolar order similar to that of prorenin (Nurun et al., 2007; Nabi et al., 2009a, 2009b). Even after 28 days of administration, fluorescent tagged handle region peptide (HRP) was recognized in the renal glomeruli and tubular lumen (Ichihara et al., 2006a; Kaneshiro et al., 2007). However, a signal of these fluorescent molecules is from the intact form of HRP or not is still arguable. This argument becomes even stronger from the findings of Leckie and Bottrill (2011). They synthesized part of prosegment sequence, RIFLKRMPSIR (it contains an additional arginine residue at the C-terminus of the decoy) and its scrambled sequence (SRRMIFPIKLR) to find out a novel binding sites in human umbilical vein endothelial cells using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Finally, they concluded that the binding of the human prorenin peptide $R^{10}IFLKRMPSIR^{20}$ to HUVEC proteins is not specific for amino acid sequence and probably involves a general peptide/protein uptake mechanism without detecting a specific prorenin prosegment binding sites (Leckie & Bottrill, 2011). Moreover, decoy peptide containing fluorescent component (carboxyfluorescein) either at N-terminus or C-terminus showed different binding affinity for (P)RR compared to that of wild type decoy *in vitro* (Nabi et al., 2010). Recombinant (P)RR coupled to CM5 sensor chips in BIAcore assay system (Nabi *et al*, 2009a, 2009b), immobilized on synthetic surfaces (Nabi *et al*, 2009b), (P)RR over expressed on COS-7 cells (Nurun *et al*, 2007) have revealed that decoy inhibits binding of renin/prorenin to (P)RR. The inhibitory constant (K_i) for the peptide was found at the nanomolar order. Also, subsequent *in vivo* studies have been carried out to show beneficial role of decoy peptide in ameliorating the end-stage organ damage related disorders by abolishing non-proteolytic activation of prorenin via inhibition of prorenin binding to (P)RR (Ichihara et al., 2004; 2006b & c; Kaneshiro et al., 2007; Satofuka et al., 2006, 2007, 2009; Wikinson-Berka et al., 2010).

Interestingly, decoy peptide has also been found to inhibit binding of renin to (P)RR and this action of decoy on renin is yet to be clarified. Based on these annotations and on the tertiary structure of renin as well as predicted tertiary structure of prorenin, the possibility of having a common site in both renin and prorenin through which these molecules can interact with the (P)RR other than the decoy peptide sequence was hypothesized. A new sequence ($S^{149}QGVLKEDVF^{158}$) that localizes in the flexible junctional region between the N- and C-domains of renin/prorenin termed as the "hinge" has recently been reported to have such pivotal role for renin/prorenin binding to (P)RR (Nabi et al., 2009b). The K_D for the binding of the "hinge" peptide to (P)RR was five times higher than that of the decoy and estimated to be 17 nmol/L. The "hinge" showed higher binding affinity to the (P)RR than that of another peptide ($A^{248}KKRLFDYVV^{257}$) from the C-domain of renin/prorenin molecule,. Like the decoy, "hinge" peptide also reduced the resonance signal of renin/prorenin binding to (P)RR as observed in BIAcore, and equilibrium state analysis revealed this paradigm as a

competitive inhibition with the K_i of 37.1 and 30.7 nmol/L, respectively (Nabi et al., 2009b). Therefore, these data suggest that not only the decoy peptide but also the "hinge" region peptide together accounted for the higher binding affinity of prorenin and hence, prorenin molecule has at least two high affinity sites while renin has a single site for their binding to (P)RR. Considering the nanomolar binding affinities of renin/prorenin and handle region peptide, Duncan J Campbell in one of his review article suggested that the (pro)renin receptor may have at least two separate binding domains, one domain is for renin and the other one is for prorenin prosegment and/or HRP (Campbell, 2008). Though, prorenin has two regions to interact with (P)RR, but to confirm the existence of different binding sites within (P)RR for its ligands, three dimensional structure of (P)RR has to be elucidated.

Activation of renin angiotensin system or in other words, generation of Ang-I by (P)RR mediated non-proteolytically activated prorenin depends on the sources of prorenin. Human prorenin showed higher binding affinity to both human and rat (P)RR compared to that of rat prorenin (Biswas et al., 2010a). More interestingly, either bound to human or rat (P)RR, molecular activity of non-proteolytically activated human prorenin was 2-4 fold higher than that of rat prorenin (Biswas et al., 2010), which could be due to the slow activation rate through change in conformation of rat prorenin compared to that of human prorenin after protein-protein interaction. Contribution of prorenin prosegment in the non-proteolytic activation mechanism was reported earlier *in vitro* (Suzuki et al., 2000). Chimera of human renin and rat prosegment showed very slow activation like native rat prorenin compared to the chimera of rat renin and human prosegment. Thus, it could be proposed that the prosegment sequence of prorenin played a pivotal role for the activation of prorenin molecules. More concisely, species specific regions within the prorenin prosegment like "handle" (Nurun et al., 2007; Suzuki et al., 2003) and decoy peptides (Nurun et al., 2007; Nabi et al., 2009a, b) actually crucial for the interaction of prorenin with (P)RR and also, for the non-proteolytic activation mediated by protein-protein interaction. Activation of rat prorenin through change in conformation at acidic condition required long time, even days to month (Suzuki et al., 2000). However, (P)RR mediated activation of rat prorenin has been observed within hours using recombinant (P)RR on *in vitro* synthetic surface system (Nabi et al., 2006; Biswas et al., 2010a) or overexpressing (P)RR on COS-7 cells (Nurun et al., 2007) or on rat VSMCs (Batenburg et al., 2007; Zhang et al., 2008). This might be the result of quick conformational change of prosegment of rat prorenin exerted by the interaction of one protein (receptor) with the other (ligand).

Furthermore, while cosidering the binding mechanism of renin and prorenin to their receptor, (P)RR has not only been discussed from the ligand's point of view, rather primary structure of (P)RR has also got similar attention for explaining the possible mechanism of receptor's involvement in ligand binding. On the other hand, though three dimensional (3D) structure of renin (Dhanaraj et al., 1992) and a predicted 3D model of prorenin (Suzuki et al., 2003; Nabi et al., 2009b) are available but due to lack of 3D structure of the receptor, mechanism for interaction of (pro)renin can not be explained from the receptor's point of view. However, several anti-(P)RR antibodies designed from the middle part (^{107}DSVANSIHSLFSEET^{121} named as anti-107/121 antibody) and C-terminus [^{221}EIGKRYGEDSEQFRD^{235} and ^{237}SKILVDALQKFADD^{250}; close to the N-terminus of transmembrane region of the receptor, named as anti-221/235 and 237/250 antibodies, respectively] regions of (pro)renin receptor have been used in many studies (Nabi et al, 2009a, 2009b; Nabi et al., 2012). Depending on the

flexibility of the anti-(P)RR antibody associated (pro)renin receptor, it would show its binding affinity towards the ligands. The calculated binding affinities of prorenin were $2.9x10^{-9}$, $1.2x10^{-9}$ and $1.74x10^{-9}$ nM, when (P)RR was immobilized or occupied by anti-107/121, anti-221/235 (Nabi et al, 2009a, 2009b) and 237/250 antibodies (Nabi et al., 2012), respectively. The recombinant (P)RR tagged with six histidine residues was synthesized in a cell free *in vitro* system using wheat germ lysate. It was hypothesized that the His tag sequence at the C-terminal end would retain the transmembrane characteristics of (P)RR *in vitro*. So, (P)RR occupied by the anti-His tag antibody would indicate its native binding pattern while interacting with the ligands. Study showed that the binding affinity of prorenin to anti-His tag antibody-bound (P)RR was 7.8 nM (Nabi et al., 2009a) and other studies using over expressed (P)RR on the cell surface showed comparable nanomolar order of binding affinity of prorenin to (P)RR (Nguyen et al., 2002; Nurun et al., 2007; Batenburg et al., 2007). Reports available so far indicate that binding region for prorenin within (P)RR resides possibly further upstream region of the amino acid residue at position 107, which could be more close to the N-terminal region(s) of the receptor.

2.1.2 Initiation of second messenger pathways

Binding of renin/prorenin to (P)RR initiates an intracellular signaling pathway that is independent of angiotensin II mediated pathway. Both renin and prorenin stimulated p42/p44 mitogen-activated protein kinase (MAPK) or ERK1/2 that leads to up-regulation of transforming growth factor-β1 release in mesangial cells, PAII, collagens, fibronectin (Huang *et al*, 2006; Huang *et al*, 2007; Sakoda *et al*, 2007) and cyclooxygenase 2 (Kaneshiro et al., 2006; Nguyen, 2006) as shown in Figure 3 (ii). Moreover, prorenin also activated p38 mitogen-activated protein kinase and simultaneously phosphorylate heat-shock protein-27 in cardiomyocytes (Sasris et al., 2006). Prorenin and renin induced activation of extracellular protein kinases (ERK) 1/2 in monocytes has also been reported (Feldt et al., 2008b). In the kidneys of diabetic mice, activation of all the three members of MAPK family including ERK, p38 and c-Jun NH_2-terminal kinase (JNK) was observed (Ichihara et al., 2006a), whereas another report (Sakoda et al., 2007) revealed activation of ERK not p38 and JNK upon activation of (P)RR via its ligand, prorenin. A protein called promyelocytic zinc finger (PLZF) has also been identified and this has been found to be associated with the cytoplasmic domain of the receptor (Schefe et al. 2006). Binding of prorenin to the receptor drives translocation of PLZF to the nucleus by stimulating P13K p85 pathway that ultimately generates short negative feedback loop to down regulate (P)RR expression [depicted in Figure 3 (iii)]. Furthermore, (P)RR is a component of the Wnt [wingless-type mouse mammary tumor virus (MMTV) integration site family] receptor complex. Wnt proteins are highly conserved secreted signaling molecules and regulators of multiple biological and pathological processes (Logan and Nusse, 2004). The signaling mechanism mediated by Wnt receptor in conjunction with (P)RR and H^+-VATPase has been explained in detail later in this chapter. The detail of the intracellular signaling pathway activated and mediated by the (pro)renin receptor has been depicted and categorically presented in Figure 3.

3. Pathophysiology of prorenin and (pro)renin receptor

Hepatocyte specific prorenin transgenic rat revealed direct pathophysiological action of prorenin. Prorenin is not activated in liver and less than 2% of the total circulating prorenin

found to be active in plasma. Diabetic subjects with microalbuminuria had very high prorenin to renin ration. Before the onset of microalbuminuria levels of prorenin begins to increase, and in conjunction with the glycated haemoglobin, the prorenin levels in plasma could be used to predict the occurrence of later microalbuminuria (Deinum et al., 1999). The circulating prorenin is responsible for developing hypertrophy of cardiomyocytes, glomerulosclerosis and atherosclerosis of small to medium sized artery, indicating elevated prorenin itself causes cardiomyopathy, glomerulosclerosis and atherosclerosis. Use of angiotensin converting enzyme inhibitors and angiotensin-II type 1 receptor blockers play protective role in end-stage organ damage in patients with hypertension and diabetes by suppressing the circulating RA system. Yet, low amount of renin activity is still evident in the plasma of these under treatment diabetic and hypertensive subjects which could ultimately be attributed to the enhanced tissue RA system. Thus, reasons behind the direct involvement of prorenin in the pathology of hypertension, diabetes and heart failure remained unclear. Receptor- associated prorenin system (RAPS), a novel phenomenon, sheds light on this direct action of prorenin. (P)RR, the new member of the RA system, has set a new perception about the physiological functions, activation mechanism and pathophysiological roles of renin/prorenin by activating angiotensin II-dependent or -independent pathways [Figures 3 (i), (ii), (iii)]. It has its own intracellular signalling pathways. Non-proteolytic activation of prorenin after interacting with (P)RR hypothesized that this activation mechanism of prorenin plays a pivotal role in the regulation of tissue RA system and end-organ damage in diabetic animals. (P)RR mRNA and protein expression are up-regulated in the hearts and kidneys of rats with congestive heart failure (Hirose et al., 2009). Thus, (P)RR in the heart can act as a capturing molecule for renin/prorenin which ultimately explain the presence of local renin-angiotensin system in heart, which can't synthesize renin. In diabetes, enhanced activity of oxidative stress and AT1 receptor are associated with up-regulation of (P)RR and this could be suppressed using AT1 receptor blocker and NADPH-oxidase activity inhibitor (Siragy and Huang, 2008). (P)RR mediated stimulation of signal cascade (depicted in Figure 3) of transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) in renal glomeruli (Huang et al., 2011) and enhancement of renal production of the inflammatory cytokines- TNF-alpha and IL-1beta, independent of the effects of renal Ang-II (Matavelli et al., 2010), contributes to the development and progression of kidney disease in diabetes. Up-regulation of (P)RR expression by high glucose is mediated by both PKC-Raf-ERK and PKC-JNK-c-Jun signaling pathways. Also, nuclear factor-κB and activation protein-1 are involved in high-glucose-induced (P)RR up-regulation in rat mesangial cells (Huang and Siragy, 2010).

At 5–6 months of age, transgenic rats over expressing the human (P)RR gene nonspecifically developed glomerulosclerosis with proteinuria by three to seven times without elevating the blood pressure (Kaneshiro et al., 2007). Transgenic rats over expressing human (P)RR gene exclusively in smooth muscle cells developed hypertension at their 7 months of age (Burckle et al., 2006). (Pro)renin receptor mediated non-proteolytically activated prorenin contributes to the development and progression of nephropathy including proteinuria and glomerulosclerosis in diabetic animals with high plasma levels of prorenin by increasing angiotensin II tissue generation (Ichihara et al., 2004). An increased Ang-I content was observed in the heart of double transgenic mice over expressing human prorenin and angiotensinogen compared to the single-transgenic mice (Prescott et al., 2002). These results indicate how prorenin contribute to the generation of angiotensin peptides locally and tissue

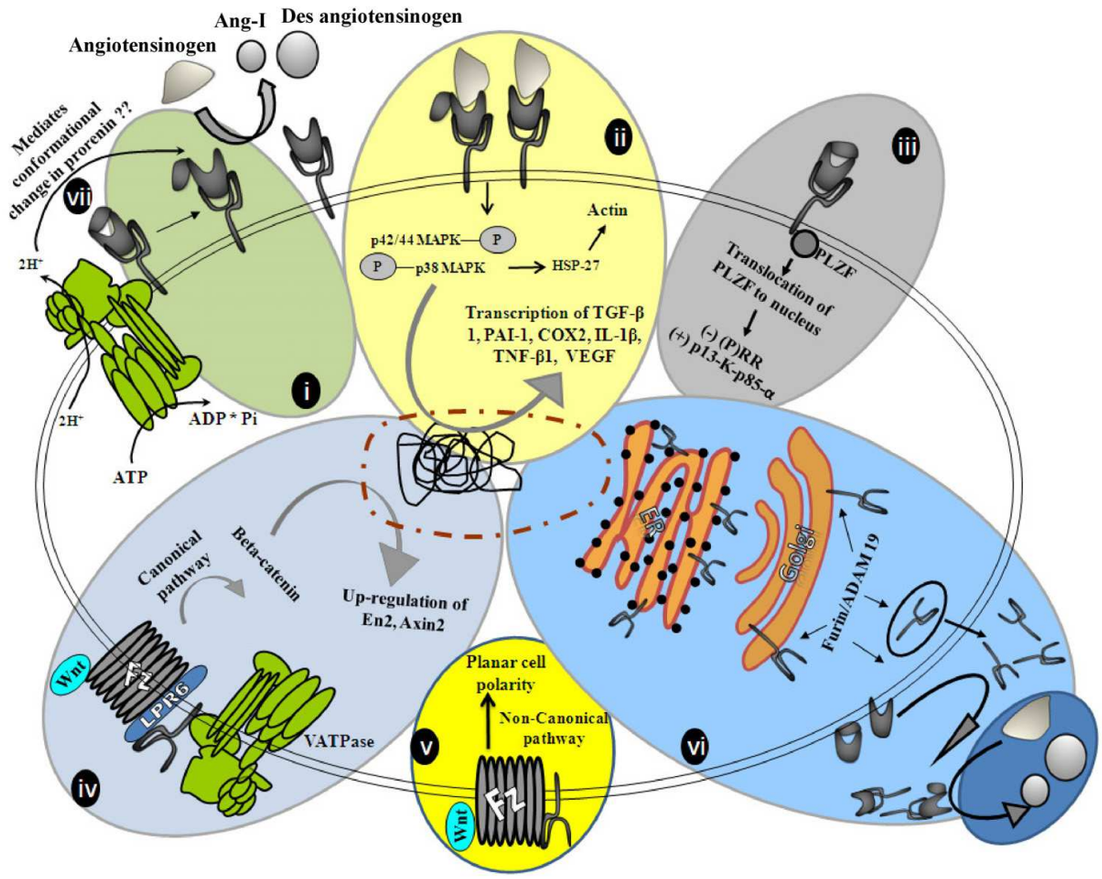

Fig. 3. Receptor associated prorenin system or RAPS mediated by prorenin and (pro)renin receptor [(P)RR] has set a new perception about the involvement of renin-angiotesin system in the pathophysiology of end-stage organ damage. Such nomenclature has been proposed due to the dual activation of tissue RAS (i) and RAS-independent signaling pathways (ii-vii). Augmentation of tissue RAS or RAPS via (P)RR initiates endocrine, paracrine or autocrine activities mediated by angiotensin II peptide. Binding of renin/prorenin to (P)RR initiates signal transduction via angiotensin II independent pathway by activating mitogen activated protein kinases (MAPKs) that induce expression of many regulatory proteins (ii). Translocation of promyelocytic zinc finger (PLZF) after prorenin binding to (P)RR leads to a short negative feed back loop that in turn suppresses (P)RR expression (iii). Also, (P)RR itself, independent of renin/prorenin, mediates Wnt-βcatenine (canonical, iv) and Ez/PCP (non-canonical) signaling pathways (v). (P)RR can be processed in the golgi apparatus by Furin or ADAM19 to its soluble form (vi). The shedded (P)RR through exocytosis can come outside of the cell and has been detected in human plasma and cultured cell medium. Shedded (P)RR binds (pro)renin. Prorenin bound to soluble (P)RR performs enzymatic activity (vi). V-ATPase participates in proton transport. C-terminal region of (P)RR is identical to the sequence of V-ATPase. This is not clear whether non-proteolytic activation of prorenin through conformational change after receptor binding is also mediated by the acidic environment created by membrane associated V-ATPase and (P)RR (vii).

damage after being taken up by tissues from circulation. (P)RR is up-regulated in kidneys of diabetic rats and renal mesangial cells exposed to high glucose concentration (Siragy and Huang, 2008; Huang and Syragi, 2010). Rapid phosphorylation at the serine residues of (P)RR in response to hyperglycemia up-regulates TGF-beta1-CTGF cascade (Huang et al., 2011), which initiates or augments kidney disease in diabetic rats. An increased Ang-I content was observed in the heart of double transgenic mice over expressing human prorenin in the liver and human angiotensinogen in the heart as compared to the single-transgenic mice (Prescott et al., 2002). These results indicate how prorenin contribute to the generation of angiotensin peptides locally and tissue damage after being taken up by tissues from circulation. Moreover, (P)RR by stimulating non-proteolytic activation of prorenin contribute to the development of renal and cardiac fibrosis in spontaneously hypertensive rats (SHRs) (Ichihara et al, 2006a,b). These data demonstrated the possible involvement of (P)RR in the pathogenesis of heart failure and kidney tissue damage. Association of (P)RR gene polymorphism with high blood pressure and left ventricular hypertrophy substantiate the important role of (P)RR in the pathogenesis of hypertension in humans (Hirose et al., 2011; Ott et al., 2011) *In vitro* and animal studies have shown that increased receptor expression could be linked to high blood pressure and to cardiac and glomerular fibrosis by activating mitogen-activated protein kinases and by upregulating gene expression of profibrotic molecules. Also, animal studies with angiotensin-II type 1a receptor deficiency showed that the (P)RR is involved in the development and progression of diabetic nephropathy through angiotensin-II independent pathway via activation of intracellular pathways (Ichihara et al., 2006c).

Association of prorenin and the (P)RR with the development of ocular pathology/diseases has been reported (Satofuka et al., 2006, 2007, 2008; Wikinson-Burka et al., 2011). Non-proteolytic activation of prorenin mediated by (P)RR is associated with retinal neovascularization in experimental retinopathy model of prematurity (Satofuka et al., 2007). Using the same model, the involvement of prorenin and (P)RR in the pathological angiogenesis, leukocyte accumulation and intracellular adhesion molecule-1 with vascular endothelial growth factor expression; retinal gene and protein expression of inflammatory mediators has also been demonstrated (Satofuka et al., 2006, 2008). RILLKKMPSV, a peptide sequence of rat prorenin prosegment, influences the vasculature, glia and neurons, and (pro)renin receptor expression in the retina (Wikinson-Burka et al., 2011).

4. Functions of (P)RR other than its involvement in renin-angiotensin system

The prototype of sequence homology between (P)RR from human and other species actually gave a clue regarding its plausible additional role in biological processes other than RAS. Two or more genes homologous to (P)RR have been found in *C. elegans* and *Drosophila melanogaster* that are phylogenetically distant from human. These species also express some components of RAS, which are not involved in homeostasis or electrolyte balance. Thus, (P)RR in these species may contribute to functions not related to RAS.

The C-terminal truncated fragment of (P)RR helps to assemble vacuolar H^+-proton adenosine triphosphatase (V-H^+-ATPase) (Ludwig et al., 1998). The (P)RR is also identical to endoplasmic reticulum–localized type 1 transmembrane adaptor precursor (CAPER) (Burckle & Bader, 2006; Campbell, 2006; Bader, 2007; Strausberg et al., 2002). Evolutionarily

V-H^+-ATPase is a highly conserved ancient enzyme in eukaryotic cells (Nelson et al., 2000) and this could be one of the most plausible reasons behind the sequence homology of C-terminus of human (P)RR with the evolutionarily close species like rat, mouse, chicken, drosophila, mosquito, zebra fish, frog and remote like *C. elegans* and bacteria *Ehrlichia chaffeensis*. (P)RR exists in truncated form composed of transmembarne region and the cytoplasmic tail that co-precipitates with V-ATPase may govern its function unrelated to RA system (Bader 2007). For this reason, (P)RR is also known as ATP6ap2 (adaptor protein type II vacuolar H^+-ATPase). The V-H^+-ATPase is expressed in the collecting ducts and distal tubules within the kidney, where it contributes to the urinary acidification as well as play pivotal role in endocytosis (Toei et al., 2010). Different subunits of V-ATPase perform different functions, notably, mutations in genes encoding C or D subunits in mice involved in embryonic lethality giving an evidence that V-ATPase plays an important role in development (Inoue et al, 1999; Miura et al, 2003), mutated B1 or A3 subunit involved in metabolic acidosis and osteoporosis in mice, respectively (Li et al., 1999; Finberg et al., 2005) and altered B1 or A4 subunit causes distal renal tubular acidosis in human (Karret, 1999; Smith, 2000). It is suggested that (P)RR and vacuolar H^+-ATPase are linked together in the kidney (Advani et al., 2009) while, for the assembly and function of vacuolar H^+-ATPase in the heart, (P)RR plays very pivotal roles (Kinouchi et al., 2010).

Recent evidences demonstrate that (P)RR is a component of the Wnt receptor complex (Cruciat et al., 2010). It is essential for *en2* expression because of its requirement in Wnt signaling. It also acts down stream of Wnts and upstream of β-catenin [Figures 3 (iv) and (v)]. Deletion of the cytoplasmic domain of (P)RR, which mediates renin signaling inside cell, showed no effect on Wnt receptor binding suggesting that (P)RR acts in a renin-independent manner as an adaptor between Wnt receptors and the V-ATPase complex. Moreover, malfunctioning (P)RR contributed to the abnormal tadpoles characterized by small heads, shortened tails, as well as defects in melanocyte and eye pigmentation at the early embryonic stage as (P)RR and V-ATPase are required to mediate Wnt signaling during antero-posterior patterning of *Xenopus*'s early central nervous system development (Cruciat et al., 2010). A homologue of (pro)renin receptor in Drosophila [d(P)RR], localized mainly to the plasma membrane, has an evolutionarily conserved role at the receptor level for activation of canonical and noncanonical Wnt/Fz (frizzled) signaling pathways [Figure 3 (v)]. Attenuation of d(P)RR affects Wg target genes in cultured cells and *in vivo* (Buechling et al., 2010). Over expressed d(P)RR interacts with Fz and Fz2 receptors which is required for planar cell polarity in Drosophila epithelia and also for convergent extension movements in *Xenopus* gastrulae. Small interfering RNAs targeting human (pro)renin receptor significantly reduced Wnt-responsive TopFlash reporter activity in HEK293T cells. Thus, (P)RR has a conserved role in mediating Wnt signaling in human (Buechling et al., 2010). This data is also consistent with the findings of Cruciat et al (2010) who demonstrated the developmental role of (P)RR in *Xenopus*. Further, asymmetric subcellular localization of frizzled, a seven-pass transmembrane receptor that acts in both wingless (Wg) and planar cell polarity (PCP), is prerequisite for the proper functioning of PCP signaling pathway (Hermle et al., 2010). It has been demonstrated that the function of VhaPRR, an accessory subunit of the vacuolar (V)-ATPase proton pump in Drosophila and also known as the VhaM8-9 because of its sequence homology with V-ATPase, is tightly associated with Fz but not to other PCP

core proteins. Fz fails to localize asymmetrically in absence of VhaPRR. It also acts as the modulators of canonical Wnt signaling pathway in larval and adult wing tissues. VhaPRR knock down caused multiple wing hair and hair mispolarization phenotypes (Hermle et al., 2010). These indicate the association of (P)RR in non-canonical (Fz/PCP) signaling pathways. Recent evidences regarding the association of (P)RR with H+-ATPase and Wnt signaling pathway shedding light on the reason behind the connection of non-proteolytic activation of prorenin by (P)RR with glomerulosclerosis, fibrosis, proteinuria. Though *in vitro* studies suggested non-proteolytic activation of prorenin mediated by (P)RR, but it is yet to determine whether the activation is mediated only by the protein-protein interaction, or by the co-operation of (P)RR and V-H^+-ATPase, or only due to the acidic environment created as a result of proton transport *in vivo* [Figure 3 (vii)]. However, because Wnt signaling pathway promotes renal fibrosis, glomerulosclerosis and proteinuria, (He et al., 2009; Dai et al., 2009) it is possible that (P)RR might act in a combination of (P)RR-H^+-ATPase-Wnt signaling pathway. Thus, (P)RR is involved in the Wnt/β-catenin canonical and Wnt/PCP non-canonical pathways in conjunction with V-H^+-ATPase in a renin-independent fashion [Figures 3 (iv) and (v)].

Using zebra fish, the important association of (P)RR and V-ATPase in the development of brain and eye at the very early stage of embryonic development has been demonstrated (Amsterdam et al., 2004). A mutation in (P)RR is very lethal that causes death before the completion of embryonic stage by creating severe malformations of the central nervous system. In fact, while ACE is required to maintain fertility and ACE2 serves as a receptor for the SARS corona virus [causing factor for severe acute respiratory syndrome (SARS)], a single amino acid mutation in exon-4 of (P)RR mRNA associated with X-linked mental retardation and epilepsy (Ramser et al., 2005), and thus, (P)RR seems to be important for brain development and cognition. Also, another major finding (Contrepas et al., 2009) stated that (P)RR play essential role in neuronal cell differentiation. Other than the embryonic development, (P)RR gene polymorphism has been found to be associated with high blood pressure in Caucasian and Japanese male subjects (Hirose et al., 2011; Ott et al., 2011). Elevated blood pressure and increased heart rate in transgenic rats over expressing (P)RR in smooth muscles have been reported in their models (Burckle et al., 2006).

5. Inhibition of the activities of the components of renin angiotensin system: (pro)renin receptor as a new therapeutic target

Peptides mimicking the structural part of prorenin prosegment (pro-enzyme of renin) or N-terminal sequence of angiotensinogen containing the renin cleavage site were the first-generation of renin inhibitors (Boger et al., 1985; Hui et al., 1987; Bolis et al., 1987). Parenteral administration of these drugs efficiently reduced blood pressure by inhibiting renin activity in animals and in human being (Boger et al., 1985; Webb et al., 1985). However, due to their peptidic nature, these drugs had very poor oral bioavailability. Later, chemically modified CGP29287 achieved more attention as renin inhibitor due to its stability and longer duration of action when given orally (Wood et al., 1985). Further, development of other drugs like enalkiren (A 64662), CGP38560A, remikiren (Ro 425892) and zankiren (A 72517) with molecular weight of a tetra-peptide (Wood et al., 1994,1989; Maibaum et al., 2003) also failed to attract attention due to their low bioavailability (<2%), a short half-life

and weak blood pressure lowering activity when administered orally (Wood et al., 1994; Nussberger et al., 2002; Rongen et al., 1995). On the other hand, an orally inactive peptide from snake venom established the important role of angiotensin converting enzyme (ACE) inhibitors in regulating blood pressure. This led to the development of Captopril, the first ACE inhibitor. Moreover, blood pressure lowering activity, to a great extent, depends on the inhibiting ability of plasma renin activity (PRA) and/or reducing plasma renin concentration (PRC). Thus, use of ACE inhibitors or angiotensin receptor blockers (ARBs) for inhibiting renin angiotensin system is not as effective as it should be because these inhibitors ultimately increase PRA or PRC (Mooser et al., 1990; Azizi et al., 2004). In addition, inhibition of ACE increases angiotensin I, which would be, via ACE-independent pathways by using cathepsins and tonins, converted into angiotensin II (Wolny et al., 1997; Hollenberg et al., 1998). Together these data indicate that direct renin inhibitors could be the superlative choice as an anti-hypertensive agent which would lower plasma renin activity.

Aliskiren, an octanamide, the first known representative of a new class of completely non-peptide, low-molecular weight, orally active transition-state renin inhibitor, that progressed to phase-III clinical trials (Wood et al., 2003). After oral dose of aliskiren (from 40 to 640 mg/day) in healthy volunteers, its plasma concentration increased dose dependently and the peak concentration reached after 3–6 hour with an average half life of 23.7 hour (Nussberger et al., 2002) making the compound suitable for once-daily administration. The oral bioavailability was 2.7%. Plasma steady-state concentrations were reached after 5–8 days of treatment. Aliskiren can inhibit enzymatic activities of receptor-bound renin and non-proteolytically activated prorenin, while it has no effect on the interaction of renin/prorenin with (P)RR. Also, aliskiren could not act as (P)RR blocker to inhibit renin/prorenin binding to (P)RR or failed to prevent (pro)renin signaling (Feldt et al., 2008b). Interestingly, when renin was incubated with aliskiren and then, allowed to bind to (P)RR, the binding affinity of renin to (P)RR decreased more than 1000 fold *in vitro* (Biswas et al., 2010b).

Also, an ideal blocker for (pro)renin receptor is indeed a necessity of time considering the direct association of (P)RR with increased blood pressure and its indirect involvement, via non-proteolytic activation of prorenin, in the pathogenesis of end-stage organ damage in hypertension, diabetes and ocular diseases. The efficacy of a peptidic blocker known as decoy peptide (R^{10P}IFLKRMPSI^{19P}) designed from the N-terminus of prorenin prosegment on the basis of the sequence of handle (I^{11P}FLKR^{15P}) region peptide was reported earlier for improving organ damage (Ichihara et al., 2004). Both human and rat decoy peptides inhibited the bindings of human and rat prorenins to their respective (P)RR expressed on the membranes of COS-7 cells with a similar K_i of 6.6 nM (Nurun et al., 2007). This peptide inhibited the bindings of not only prorenin but also renin to the preadsorbed receptors with the K_i values of 15.1 and 16.7 nM, respectively (Nabi et al., 2009b). Moreover, real-time bindings using surface plasmon resonance (SPR) technique in BIAcore assay system revealed evidence for the direct binding of native decoy peptide to the immobilized (P)RR with K_i of 3.5 nM (Nabi et al., 2009a, 2009b). The SPR technique displayed reduced resonance signal of prorenin binding to (P)RR while co-incubated with the decoy peptide.

The decoy proposition has also been tested *in vivo* using various transgenic models. Administration of HRP significantly inhibited increased levels of renal angiotensin II, the development of proteinuria and glomerulosclerosis in a model of diabetic nephropathy; rat

HRP completely prevented the development of diabetic nephropathy in heminephrectomized streptozotocin induced diabetic rats without affecting hyperglycemia (Takahashi et al., 2007). Urinary albumin excretion and the renal production of tumor necrosis factor-α and interleukine-β1 were decreased significantly when rat HRP was given directly into the renal cortical interstitium of diabetic rats (Matavelli et al., 2010). Prevention of the development of proteinuria, glomerulosclerosis, and complete inhibition of the activation of ERK1/2, p38, JnK in the kidney of diabetic angiotensin-II type-1a receptor-deficient mice was reported and thus, the role of (P)RR via angiotensin II independent pathway in association with prorenin was suggested (Ichihara et al., 2006c). Other investigators also confirmed the action of prorenin and (P)RR via angiotensin-II independent pathway (Huang et al., 2006; Muller et al., 2008; Feldt et al., 2008a, b). Moreover, HRP inhibits the development of retinal neovascularization by inhibiting non-proteolytic activation of prorenin caused by interaction with (P)RR in experimental retinopathy model of prematurity (Satofuka et al., 2007). Satofuka *et al.* using the same model, showed that the HRP suppressed the pathological angiogenesis, leukocyte adhesion and retinal expression of ICAM-1 and VEGF; also, reduced retinal gene and protein expression of inflammatory mediators (Satofuka et al., 2006, 2009). HRP also improved vascular disorder in a model of retinopathy of prematurity, but had detrimental effects on retinal neurons and glia. These effects occurred despite HRP not being detected in plasma. In young spontaneously hypertensive rats (SHR) under high salt-diet, HRP not completely but significantly attenuated glomerulosclerosis with proteinuria, cardiac hypertrophy with left ventricular fibrosis without affecting the development of hypertension (Ichihara et al, 2006 a, b). In addition, Susic et al made a further interesting observation by reporting reduced beneficial effects of decoy (PRAM-1) in SHR rat with normal diet (Susic et al., 2008).

On the contrary, many researchers are not satisfied about decoy's role as a fruitful (P)RR blocker (Batenburg et al., 2007; Muller et al., 2008; Feldt et al., 2008a, b; Mercure et al., 2009). Chronic HRP treatment did not improve target organ damage in renovascular Goldblatt hypertensive rats with high renin, prorenin and PRA that lead to Ang-II dependent target organ damage rather HRP counteracts the beneficial effects of aliskiren (van Esch et al., 2011). Also, HRP had no effects on the activation of signal transduction mediated by prorenin-(P)RR interaction (Feldt et al., 2008a). On the other hand, very recently, (P)RR siRNA technique and prolonged use of HRP or valsartan showed inhibition of rapid phosphorylation in the serine residues of (P)RR that ultimately suppressed inflammation in the kidneys (Huang et al., 2011). The concentration of HRP could not be measured in both blood and plasma of rats infused with either 0.1 or 1 mg/kg HRP per day, which suggested rapid metabolism of the peptide *in vivo* and this interpretation was supported by the finding that HRP was metabolized with a half-life of 5 minutes in EDTA-plasma at 37°C (Wikinson-Burka et al., 2011). Recycling of (P)RR between the cellular compartments and cell surface has been demonstrated earlier (Batenburge et al., 2007). Later, this annotation has been experimentally proved by the action of furin (Cousins et al., 2009) and ADAM19 (Yoshikawa et al., 2011), which till-to-date could be one of the most appropriate and acceptable explanation behind the useful execution of "decoy" as (P)RR blocker in some animal model or cell line while in other models, the "decoy" is not effective even at the same or sometimes higher concentration.

6. Conclusion and future direction

A sensitive enzyme-linked immunosorbent assay has been established to detect the level of soluble (P)RR in the medium of cultured cells and also in cell lysates (Kazal et al., 2011). It is now very important to set up such easily pursuable and sensitive method for the detection of (P)RR in human plasma. It may facilitate to diagnose specific disease or to measure degree of organ damage or to predict the end-stage organ damage. Three dimensional structure of (pro)renin receptor has to be resolved to clear the ambiguity of decoy hypothesis, to find out the binding site(s) of prorenin, renin and the decoy peptide within the molecule. Furthermore, a well accepted (P)RR blocker is now the demand of time to reduce the effects of (P)RR on end-stage organ damage. Thus, (P)RR, now-a-days, should be the novel target for developing new therapeutic approaches to ameliorate end-stage organ damage related disorders. However, considering the involvement of (P)RR in organ development specially in eye and brain development, more extensive studies should be performed before designing a (P)RR blocker.

7. References

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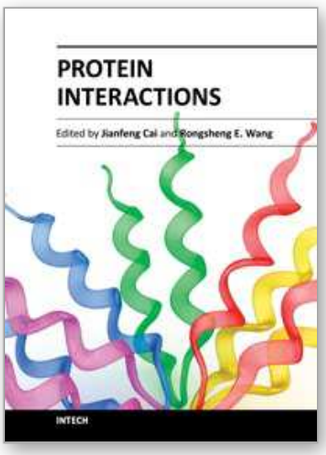

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Protein interactions, which include interactions between proteins and other biomolecules, are essential to all aspects of biological processes, such as cell growth, differentiation, and apoptosis. Therefore, investigation and modulation of protein interactions are of significance as it not only reveals the mechanism governing cellular activity, but also leads to potential agents for the treatment of various diseases. The objective of this book is to highlight some of the latest approaches in the study of protein interactions, including modulation of protein interactions, development of analytical techniques, etc. Collectively they demonstrate the importance and the possibility for the further investigation and modulation of protein interactions as technology is evolving.

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