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REVIEW

Potential cross-talk between (pro)renin receptors and Wnt/frizzled receptors in cardiovascular and renal disorders

Pitchai Balakumar[1] and Gowraganahalli Jagadeesh[2,3]

The renin–angiotensin system and Wnt/frizzled receptor signaling pathways are important in the development of essential organs, and their aberrant activation results in cardiovascular and renal pathologies. The (pro)renin receptor ((P)RR)-bound (pro)renin is enzymatically active generating angiotensin-II and activating mitogen-activated protein kinases, leading to cell proliferation and to upregulation of profibrotic genes expression, resulting in end-organ damage. The (P)RR does more than bind to (pro)renin, because it is functionally linked to the vacuolar-H^+-ATPase (v-H^+-ATPase) that regulates pH of cellular and intracellular vesicles, and to Wnt signaling. This signaling pathway is essential for cell survival, embryonic development and has had a role in various disease states as evidenced by mutation or genetic ablation of the (P)RR gene. This suggests two types of functions of (P)RRs, first one as a receptor for (pro)renin and second one as an accessory subunit of the v-H^+-ATPase and a cofactor of the Wnt/Fz receptor complex. This review will discuss both of these functions of (P)RRs thereby giving new perspectives as to the roles of (P)RRs in cardiovascular and renal pathologies.
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INTRODUCTION

Wnt signaling is an essential molecular pathway that modulates cell–cell communication, cell behavior, cell proliferation, asymmetric cell division, cell polarity, survival and neural patterning in both embryo and adult humans.[1,2] It is a prerequisite for normal development of cardiovascular[3–5] and renal systems.[6] It is considered both a friend and foe, because abnormal Wnt signaling has been implicated in several physiopathologic conditions, including congenital malformations, cancer, osteoporosis, degenerative disorders and aging.[2,3,7,8] Defective Wnt signaling is also implicated in the pathogenesis of cardiovascular and renal abnormalities.[3,9,10]

Wnts are secreted glycoproteins of several amino acids in size and contain large cysteine-rich domains. There have been around 20 Wnt isoforms identified in humans. The Wnt family members can be divided into two distinct subfamilies on the basis of their ability to induce transformation of a mouse mammary epithelial cell line: (a) the highly transforming Wnt proteins known as the Wnt-1 subfamily, including Wnt-1, Wnt-3, Wnt-3a and Wnt-7a; (b) the intermediate/non-transforming Wnt proteins known as the Wnt-5a subfamily, including Wnt-2, Wnt-4, Wnt-5a, Wnt-5b, Wnt-6, Wnt-7b and Wnt-11.[3] Wnt proteins interact with frizzled (Fz) receptors, which are seven-transmembrane segmented, heterotrimeric guanine nucleotide-binding protein-coupled receptors with a characteristic large extracellular N-terminus.[11] Additionally, Wnt proteins interact with several co-receptors such as the LRP5 and LRP6 (low-density lipoprotein receptor-related protein 5/6) family of low-density lipoprotein receptors, and the Ror and Ryk family of tyrosine kinase receptors.[9,12–14]

The Wnt/Fz pathways may be categorized into the canonical (Wnt/β-catenin or Wnt 1 class) pathway, and the non-canonical (β-catenin-independent or Wnt 5A class) pathway (the Wnt/JNK or Wnt/planar cell polarity pathway (Wnt/JNK/PCP) and the Wnt/Ca^{2+} pathway).[2,12] The canonical Wnt/β-catenin pathway involves the stabilization of cytoplasmic β-catenin, which then enters the nucleus and activates a specific gene transcription program.[2] On the other hand, on Wnt/Fz signaling, the non-canonical PCP pathway activates C-Jun N-terminal kinase (JNK) and Rho-kinase signaling cascade resulting in remodeling of the cytoskeleton and changes in cell adhesion and motility.[15,16] Whereas the requirement of G proteins for non-canonical Wnt signaling was known for a long time,[17] only recent studies have demonstrated the novel role of G proteins in canonical Wnt signal transduction. The Fz receptors are coupled to $G_{\alpha o}$ and $G_{\alpha q/11}$ for canonical signal transduction and $G_{\alpha o}$, $G_{\alpha q/11}$, $G_{\alpha i}$ and $G_{\alpha t}$ (and $G_{\beta\gamma}$ dimers) for non-canonical signal transduction.[18–21]

[1]Cardivascular Pharmacology Division, Department of Pharmacology, Institute of Pharmacy, Rajendra Institute of Technology and Sciences (RITS), Sirsa, India and [2]Division of Cardiovascular and Renal Products, Center for Drug Evaluation and Research, US Food and Drug Administration (FDA), Silver Spring, MD, USA
[3]The opinions expressed herein are those of GJ and do not necessarily reflect those of the US Food and Drug Administration.
Correspondence: Dr G Jagadeesh, Division of Cardiovascular and Renal Products, Center for Drug Evaluation and Research, US Food and Drug Administration (FDA), 10903 New Hampshire Avenue, Building 22, Room 4128, Silver Spring, MD 20993-0002, USA.
E-mail: gowra.jagadeesh@fda.hhs.gov
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In all of these pathways, Wnt ligands bind to Fz receptors and transduce signals via heterotrimeric G proteins to the multifaceted cytoplasmic protein known as dishevelled (Dvl). The latter is the focal point from which the signaling pathways diverge and relay signals to downstream effectors. A direct interaction between Fz receptors and Dvl has not been shown, and the mechanism by which Dvl distinguishes different Wnt pathways has not been delineated. At present, it is not clear which Wnt ligands activate which Fz receptors (currently there are 10 known) or whether all receptors couple to both the canonical and non-canonical pathways.[5,22,23]

The renin–angiotensin system (RAS), like the Wnt/Fz signaling system, is an imperative hormonal system involved in the development of many organs.[24] It has a pivotal role in the maintenance of vascular tone and cardiac function. The pharmacologic interruptions of aberrant activation of the RAS at different stages in its signaling cascade have been well known to be effective in treating various cardiovascular disorders. In the RAS signaling cascade, prorenin and renin are the initial players, while angiotensin-II (Ang-II), a major autocoid, is a final effector peptide. The discovery of the (pro)renin receptor ((P)RR)[25] has expanded the horizon of the RAS, as this receptor binds both renin and prorenin, triggering Ang-II-dependent and -independent signaling (p42/p44 mitogen-activated protein kinase (MAPK) activation) cascades.[26,27] This has, in fact, opened up a novel pharmacologic target for managing hypertension and end-organ damage.

Very recently, Cruciat *et al.*[28] characterized the (P)RR as a multifunctional protein that associates with vacuolar H^+-ATPase (v-H^+-ATPase) (independently of renin) and is required for canonical Wnt/β-catenin signaling and functioning. The findings of Buechling *et al.*[29] and Hermle *et al.*[30] furthermore extended the role of (P)RR in bridging canonical and non-canonical Fz complexes with v-H^+-ATPases, thereby providing a novel mechanism to regulate Wnt/Fz signaling, which are essential for adult and embryonic stem cell biology, for embryonic development, and for diseases such as cancer.[31] The (P)RR acting as a critical component of the v-H^+-ATPase has additional functions besides binding to (pro)renin, generating Ang peptides and triggering phosphorylation of MAPKs. This could have potential benefits in exploring novel pharmacological target sites, because both the (P)RR and Wnt/Fz signaling pathways are implicated in cardiovascular and renal diseases and are essential for cell survival and embryonic development. In light of this view, we, in this review, initially summarize the current knowledge on canonical and non-canonical Wnt and (P)RR signaling, and later discuss the potential mechanistic role of the (P)RR associated with the v-H^+-ATPase in Wnt/Fz signal transduction in cardiovascular and renal disorders.

CANONICAL WNT PATHWAY

In the canonical Wnt/β-catenin pathway, binding of a Wnt ligand to the Fz receptor and co-receptor LRP5/6 activates cytoplasmic protein Dvl. The Dvl protein possesses three conserved domains, an amino terminal DIX domain of 80 amino acids, a central PDZ domain of about 90 amino acids and a carboxyl-terminal DEP domain of 80 amino acids.[32] The DIX and PDZ domains of Dvl mediate canonical Wnt signaling, whereas the DEP domain is required for planar polarity signaling.[33] Thus, Dvl acts as a positive regulator of the Wnt pathway. The Dvl protein appears to be a key signaling molecule in the Wnt pathway to read signals from the plasma membrane and route them to intracellular signaling components. The activated Dvl suppresses the degradation of β-catenin, which accumulates in the cytoplasm and subsequently enters the nucleus to regulate the expression of numerous downstream genes (Figure 1). In the absence of a Wnt ligand, the glycogen synthase kinase-3β (GSK-3β) and casein kinase from the multimeric protein destruction complex (composed of adenomatous polyposis coli, axin, GSK-3β and casein kinase 1) sequentially phosphorylate the amino terminal region of β-catenin, which, as a result, undergoes a proteasome-mediated degradation in the cytoplasm.[34,35] On the other hand, the binding of Wnt to both Fz receptor and LRP5/6 stabilizes β-catenin (that is, inhibits β-catenin degradation) by inducing phosphorylation of GSK-3β-mediated inhibition of destruction complex. Moreover, a pool of axin-bound GSK-3β translocates to the membrane involving Dvl that may contribute to phosphorylation of cytoplasmic PPPSPxS motifs in the LRP6 at the cell membrane. This activity is blocked by β-adrenergic receptor kinase.[21] Binding of the axin–GSK-3β complex to phosphorylated LRP6 inhibits GSK-3β activity allowing cytoplasmic β-catenin to stabilize.[36,37] Thus, GSK-3β in the destruction complex promotes β-catenin degradation, whereas GSK-3β-mediated phosphorylation of LRP6 at the plasma membrane in response to Wnt binding to Fz receptors contributes to the stabilization of β-catenin. Taken together, Wnt binds to Fz-LRP5/6 complex and induces Fz recruitment of Dvl, which in turn recruits the axin–GSK-3β complex to the membrane, and thereby promotes LRP5/6 phosphorylation to initiate an active β-catenin signaling. The elevated cytoplasmic β-catenin enters the nucleus in a concentration-dependent manner[38] to initiate a transcriptional program with T-cell factor and lymphoid enhancer factor family members and forms a transcriptional activator complex.[8,39,40] The activator complex targets several genes such as *AXIN2*, *fibronectin*, *mab-5*, *endothelin-1*, *cyclin D1*, *Prop1*, *Oct-3/4*, *Pitx2*, *MMP7*, *EPHB*, *c-Myc*, *FGF9*, *RUNX2*, *MITF*, *BMP4*, *Dickkopf1*, *MET*, *ID2*, *T-cell factor-1*, *SOX9*, *NT-3*, *VEGF* and *TIAM1*, resulting in regulation of numerous cellular processes, including proliferation, differentiation, transformation, migration, adhesion, hypertrophy, angiogenesis, and so on.[2,38,41]

GSK-3β has a powerful antihypertrophic effect. It is inhibited by the activation of the Wnt/Fz pathway, growth factors and hypertrophic stimuli such as $G_{\alpha q}$-coupled signaling, which activates phospholipase C-β and calcineurin. The latter dephosphorylates nuclear factor of activated T cells (NFAT), which then translocates to the nucleus to activate numerous hypertrophic genes[9] (Figure 2). NFAT transcription factors have been shown mediating cardiac hypertrophy and function as primary calcineurin effectors in the heart.[42] GSK-3β is a serine/threonine protein kinase that mediates the addition of phosphate molecules on certain cellular substrates, counteracts the activity of calcineurin by phosphorylating NFAT and causing its nuclear export[9,10] (Figure 1). As a result of an active GSK-3β has a negative effect on downstream signaling mechanisms pertaining to hypertrophic gene expression, it serves as a novel therapeutic approach for cardiac hypertrophy.[9,43]

It is worth mentioning that the transcriptional activity of β-catenin may also occur independent of the activation of Wnt and Fz. Activation of G protein-coupled receptors such as prostanoid (EP2, EP4),[44] lysophosphatidic acid (LPA2, LPA3),[18,45] gonadotrophin releasing hormone[46] and parathyroid[47] can also turn on β-catenin-mediated gene transcriptional programs. In a recent study, it has been shown that the free G_α and $G_{\beta\gamma}$ subunits released from an activated G protein act co-operatively to inhibit β-catenin degradation and subsequently activate β-catenin-mediated transcriptional program.[21] The $G_{\beta\gamma}$, in concert with Dvl, recruits and activates its effector, GSK-3β to the membrane. The membrane associated GSK-3β; either alone or in association with axin, phosphorylates LRP6. The direct G_α signaling (that activates protein kinase A, B or C) was shown to inhibit GSK-3β, resulting in stabilization of cytosolic β-catenin and its subsequent nuclear translocation.[9,18,19,21]

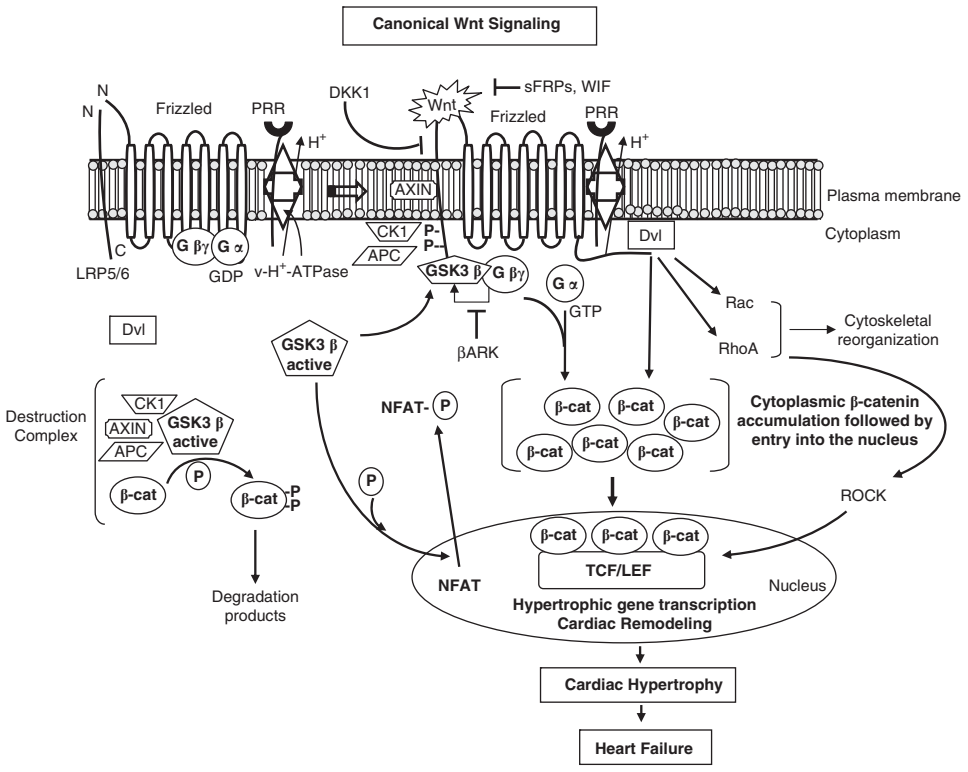

Figure 1 (Pro)renin receptor ((P)RR) regulating canonical Wnt signal transduction in cardiac hypertrophy. Off state (left): in the absence of a Wnt ligand binding to a receptor complex (consisting of frizzled receptor, G proteins, co-receptors low-density lipoprotein receptor-related protein 5/6 (LRP5/6), (P)RR/vacuolar H^+-ATPase (v-H^+-ATPase)), cytoplasmic dishevelled (Dvl) remains inactive. As a result of this, β-catenin sequestered within the destruction complex (consisting of axin, adenomatous polyposis coli, casein kinase 1 and glycogen synthase kinase-3β (GSK-3β)) is phosphorylated. This leads to the degradation of β-catenin by the proteasome. Activated GSK-3β counteracts the activity of calcineurin by phosphorylating nuclear factor of activated T cells (NFAT) and inhibiting nuclear shuttling of NFAT. On state (right): binding of a Wnt ligand to the receptor complex activates G proteins and Dvl and inhibits destruction complex through recruitment of its components to the membrane. This process facilitates cytoplasmic stabilization and nuclear translocation of β-catenin where it functions as important mediators of hypertrophic gene expression. The G_α subunit is also implicated in dissociation of the β-catenin destruction complex. $G_{\beta\gamma}$ subunit recruits and activates GSK-3β to the membrane. Dickkopf-1, soluble Fz-related proteins and Wnt-inhibitory factor-1 are extracellular inhibitors of Wnt/Fz β-catenin signaling. The membrane recruited Dvl also stimulates small GTPases RhoA and Rac resulting in a rearrangement of the cytoskeleton, and through the stimulation of Rho-kinase (ROCK), potentiates the β-catenin-dependent induction of target genes leading to cardiac hypertrophy and heart failure.

The contribution of Wnt-triggered small GTPase activation to non-canonical (β-catenin-independent) signaling is extensively studied (discussed later), but its contribution to canonical signaling is poorly documented. A recent study by Rossol-Allison *et al.*[48] in mesenchymal stem cells demonstrated that the binding of Wnt-3A to Fz and LRP5/6 activates the cytosolic Dvl, leading to activation of RhoA. The activated RhoA stimulates Rho-kinase to potentiate β-catenin-mediated gene transcription, although the precise mechanism remains unclear (Figure 1). In this study, the investigators suggest that RhoA activation does not affect β-catenin stabilization and nuclear accumulation; nevertheless, RhoA is a possible modifier of the canonical Wnt-3A-stimulated, β-catenin-dependent transcriptional program.[48]

Antagonism of canonical Wnt signaling occurs by disrupting the formation of a ternary complex consisting of Fz receptor, LRP5/6 and the Wnt ligand.[49,50] This process facilitates degradation of β-catenin by GSK-3β.[51,52] The two major classes of Wnt antagonists, soluble Fz-related protein and Dickkopf family of secreted proteins, antagonize Wnt signaling. The soluble Fz-related protein class (consisting of the soluble Fz-related protein family, the Wnt-inhibitory factor-1 and cerberus) binds directly to Wnt proteins, and alters their ability to bind to the Wnt receptor complex.[53] The Dickkopf class of antagonists (for example, sclerostin and Dickkopf-1) inhibit LRP 5/6 co-receptor activity (Figure 1) and diminish the number of Wnt co-receptors available for signaling induction.[54] Recently, the Hippo pathway has emerged as a critical regulator of Wnt/β-catenin signaling. It has been shown that the Hippo pathway inhibits Wnt/β-catenin signaling by promoting TAZ (transcriptional coactivator with PDZ-binding motiff)-mediated inhibition of phosphorylation of Dvl protein. Accordingly, abrogation of TAZ levels enhances Wnt-stimulated Dvl phosphorylation, followed by β-catenin-mediated gene transcription in the nucleus.[55] Inhibition of canonical Wnt signaling might result in activation of non-canonical JNK signaling (see below for details), as shown in several studies.[56,57]

NON-CANONICAL WNT PATHWAY

Non-canonical Wnt signaling activities include Wnt/JNK/PCP pathway and Wnt/Ca^{2+} pathway. Both of them are β-catenin-independent pathways and require heterotrimeric G proteins for Fz function. Once activated, Fz receptors engage G proteins, which recruit Dvl to the cell membrane and activate phospholipase C to initiate signaling (Figure 2). As a result of the involvement of certain proteins in Wnt/JNK/PCP and Wnt/Ca^{2+} pathways, it is suggested that they may be components of the same signaling network, rather than two distinct

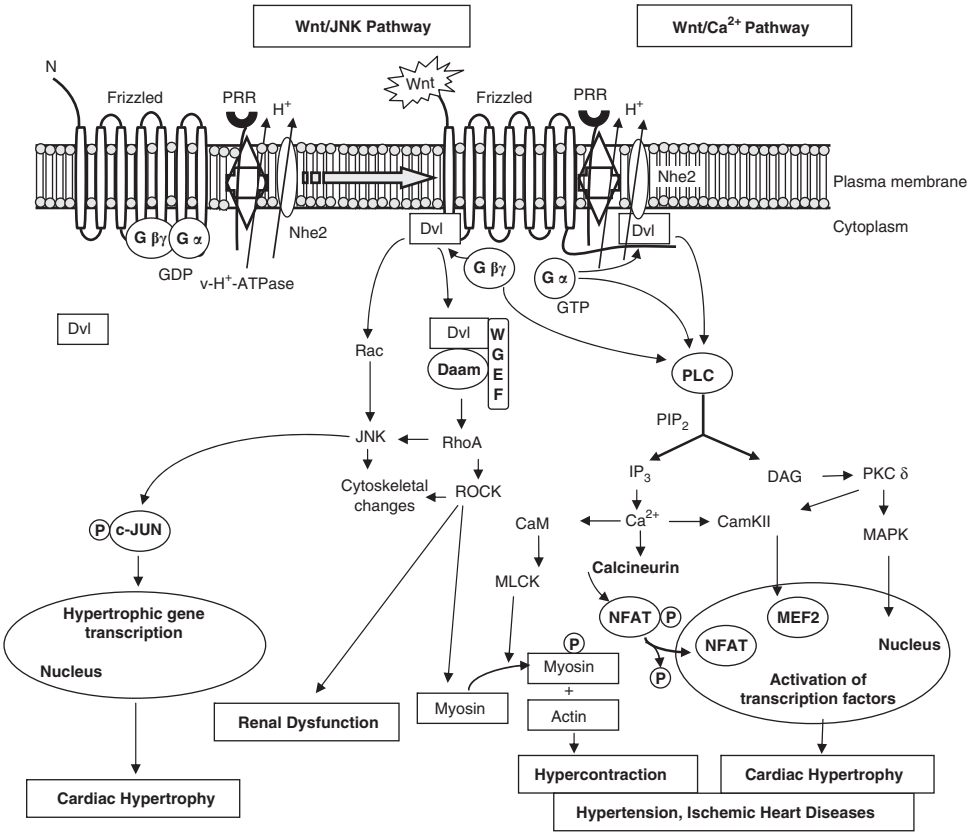

Figure 2 (Pro)renin receptors ((P)RRs) regulating non-canonical β-catenin-independent Wnt signal transduction pathways in the development of cardiovascular and renal abnormalities. Off state (left): in the absence of a Wnt ligand binding to a receptor complex (consisting of frizzled receptor, G proteins, (P)RR/vacuolar H^+-ATPase (v-H^+-ATPase), Nhe2), cytoplasmic dishevelled (Dvl) and small GTPases remain inactive. On state (right): The C-Jun N-terminal kinase (JNK)/planar cell polarity (PCP) pathways are shown on the left, whereas Ca^{2+} pathways are shown on the right. Binding of a Wnt ligand to the receptor complex activates G proteins and recruits Dvl to the membrane to initiate signaling. One branch (left) of the non-canonical involves activation of small GTPases (RhoA and Rac), which in turn activate JNK and Rho-kinase (ROCK) to regulate the actin cytoskeleton and polarity. Both JUN and ROCK functions as important mediators of cardiac hypertrophy and renal dysfunction. The second branch (right) activates Ca^{2+}/protein kinase C (PKC) signaling pathways. Both G proteins and Dvl activate phospholipase C (PLC) that generates inositol triphosphate (IP_3) and diacylglycerol (DAG). These in turn, increase intracellular Ca^{2+} that leads to the activation of CaM, calmodulin-dependent protein kinase II (CamKII), calcineurin, PKC, mitogen-activated protein kinase (MAPK) and activation of transcription factors nuclear factor of activated T cells (NFAT), myocyte-specific enhancer factor 2, which regulate cardiac development and differentiation-specific gene expression.

pathways.[2] Non-canonical Wnt signaling has been shown to inhibit canonical Wnt signaling through several mechanisms.[58]

WNT/JNK/PCP PATHWAY

PCP signaling refers to the polarization of cells in an epithelial sheet, and it may occur during gastrulation, an early phase in the development of most animal embryos.[59] In the Wnt/JNK/PCP pathway, the Wnt signal occurs via Fz in the absence of LRP5/6 co-receptor. The G protein, which recruits the modular protein Dvl to the membrane, is activated in response to the binding of Wnt to Fz. The interaction between Fz and Dvl is partly the result of a direct binding of the PDZ domain of Dvl to a conserved motif in the C-terminal region of Fz.[60,61] The multifaceted protein Dvl activates small GTPases of the Rho family including Rho, Rac and Cdc42.[15,16,62] The activated Dvl interacts with the inactive form of Dvl-associated activator of morphogenesis 1 via its PDZ and DEP domains to form Dvl–Dvl-associated activator of morphogenesis 1 complex, which interacts with the Rho guanine nucleotide exchange factor weak-similarity guanine nucleotide exchange factor, leading to activation of Rho-GTPase.[63,64] The activated Rho-GTPase subsequently activates Rho-kinase, which remodels the cytoskeleton.[15,65] Activation of Rac does not need Dvl-associated activator of morphogenesis 1; the Dvl-activated Rho and Rac further activate JNK[66] (Figure 2). The Wnt/JNK/PCP pathway regulates changes in the actin cytoskeleton required for cell shape changes and motility,[64,65] cell polarity and movements during *Xenopus* gastrulation[15] and dendrite growth in cultured hippocampal neurons.[67]

WNT/CA^{2+} PATHWAY

The Wnt/Ca^{2+} pathway may function as a critical modulator of both canonical and Wnt/JNK/PCP pathways.[1] Wnt/Fz (specifically Wnt-5a, Wnt-11)[68,69] activates phospholipase C via the trimeric G protein, resulting in the generation of diacylglycerol and inositol triphosphate. The latter increases intracellular Ca^{2+} levels and Ca^{2+} fluxes. This in turn activates numerous downstream calcium-sensitive enzymes such as protein kinase Cδ and Ca^{2+}/calmodulin-dependent protein kinase II.[23,70,71] The elevated levels of Ca^{2+} also activate the calcium-sensitive phosphatase, calcineurin, which dephosphorylates NFAT, a cytoplasmic transcription factor. On stimulation, NFAT translocates to the nucleus to activate target genes[7] (Figure 2). The NFAT signaling is

crucial for normal heart valve and vascular development during embryogenesis.[72] However, in the adult it is an important mediator of cardiac hypertrophy.[9,73] Wnt-5a-mediated non-canonical signaling has been reported to regulate human endothelial cell proliferation and migration.[74] Additionally, this pathway is suggested to have a role in inflammatory angiogenesis and vascular remodeling.[75]

IMPLICATIONS OF WNT SIGNALING IN CARDIAC PATHOLOGIC CONDITIONS

Wnt signaling is essential for early stage cardiac development including cardiac specification, morphogenesis, cardiac valve formation and establishment of the conduction system.[4,76] However, in the adult heart, the chronic activation of Wnt signaling in response to pathological stimulus may cause cardiac abnormalities especially cardiac hypertrophy and heart failure.[3,9,77] On chronic stress stimulus, the heart undergoes a remodeling event followed by reduced contractile function as a result of maladaptive cardiac hypertrophy accompanied by alteration in cardiac geometry, architecture, loss of myocytes and cardiac fibrosis (reviewed in Balakumar and Jagadeesh[10]). The signaling mechanisms involved in cardiac hypertrophy are incompletely understood, and recent evidences strongly implicate the role of Wnt signaling in cardiac remodeling and maladaptive cardiac hypertrophy.[78] On stimulation, Wnt/Fz pathway activates Dvl protein, which inhibits GSK-3β resulting in the cytoplasmic accumulation of β-catenin. This is followed by translocation of β-catenin molecules from cytoplasm to nucleus, where they induce a key event of transcription of several hypertrophic genes in the myocardium.[79] Interestingly, interruption of Wnt signaling in Dvl knockout mice attenuated the onset of pressure overload-induced cardiac hypertrophy.[77] Following experimental myocardial infarction in the mouse, β-catenin depletion attenuated post-infarct left ventricular remodeling, and significantly improved left ventricular function and survival.[80] Huang *et al.*[81] demonstrated an association between Wnt signaling and post-infarct cardiac remodeling in aged mice. The cardiac detrimental role of Wnt/β-catenin signaling was further confirmed by the fact that β-catenin knockout attenuated phenylephrine-induced hypertrophy and upregulation of fetal genes in cardiomyocytes.[82] Malekar *et al.*[83] have recently shown in aortic banded rats that Wnt signaling accelerated myocardial remodeling, and was critical for inducing maladaptive cardiac hypertrophy. In this study, the investigators observed that cardiac-specific overexpression of Dvl-1 protein increased in the stable phase of cardiac hypertrophy and eventually led to severe cardiomyopathy. The investigators provided convincing evidence that both canonical and non-canonical Wnt signaling pathways are involved in cardiac hypertrophy.[83] Taken together, the sustained activation of Wnt signaling pathway has a pivotal role in adult cardiac remodeling, and selective inhibition of Wnt signaling could serve as a promising target for the treatment of cardiac hypertrophy and heart failure.

IMPLICATION OF (P)RRS IN PATHOLOGIC CONDITIONS

One of the approaches to the treatment of hypertension, which may be considered as a major scientific advancement, involves the use of drugs targeting the RAS. The RAS is a dual hormonal system, serving as a circulating and a local tissue hormonal system as well as a central neuromodulatory system. This is supported by the fact provided by Laragh,[84] who noted that hypertensive patients exhibited a wide distribution in plasma renin activity. Pharmacologic interruption of the RAS was initially employed in the late 1970s with the advent of the Ang-converting enzyme inhibitor, captopril. As the roles of the RAS in the pathophysiology of several diseases were explored, so did the realization of the importance of inhibiting the actions of Ang-II, the key product of the RAS. This was accomplished with the introduction of Ang-II type 1 receptor antagonist, losartan, in 1995. This opened up new vistas in understanding the additional biological effects of Ang-II. The Ang-II type 1 receptor blockade may be considered near to complete blockade of RAS. However, negative feedback of circulating Ang peptides elicit rise in plasma renin activity and plasma renin concentration with both strategies blocking the RAS. Since renin is the first and rate-limiting step of the RAS, interruption of the generation of Ang-II by renin inhibitors has been suggested to provide an efficient RAS inhibition. Similarly, aliskiren, a selective renin inhibitor, blocks the enzymatic conversion of angiotensinogen to Ang-I. This demonstrated target organ protection in a double transgenic rat model of high human renin hypertension[85] but failed to protect against diabetic nephropathy and retinopathy, and cardiac fibrosis.[86–88] The discovery of the (P)RR, a specific receptor for renin and its precursor protein prorenin as suggested in the previous section, has shifted the paradigm in understanding new roles of RAS in the development and progression of cardiovascular and renal complications.

The differential in circulating concentrations of renin and prorenin and the type of activation of prorenin have a key role in the local RAS. The precursor prorenin is activated by two biological processes[26]: (a) irreversible proteolytic activation by enzymes such as proconvertase-1 and cathepsin to remove the prosegment forming an active renin; and (b) reversible non-proteolytic activation to unfold the prosegment from the enzymatic cleft through binding to the (P)RR. The latter, in addition to cleaving angiotensinogen to Ang-I, triggers the phosphorylation of extracellular signal-regulated kinase 1/2 of MAPKs and release of transforming growth factor-β, resulting in the upregulation of plasminogen activator inhibitor-1, fibronectin and collagen 1, which are hypertrophic and profibrotic signals inducing end-organ damage. In cardiomyocytes, (pro)renin-bound (P)RR stimulates p38 MAPK, heat shock protein 27 and phosphatidylinositol-3-kinase-85.[89–94] Thus, diabetic nephropathy and retinopathy, and cardiac fibrosis develop mainly by the non-proteolytic activation of (pro)renin bound to the (P)RR. An upregulation in (pro)renin and (P)RR levels contributing to the development of nephropathy in diabetic animals has been reported by many investigators (reviewed in Balakumar and Jagadeesh[26]). Recently, Matavelli *et al.*[95] extended these mechanisms suggesting that the renal (pro)renin and (P)RR axis promotes diabetic nephropathy by significantly increasing the amounts of pro-inflammatory cytokines. The handle region peptide (HRP), a putative blocker of the (P)RR, could efficiently block glomerulosclerosis,[86,88,95,96] while RAS inhibitors were unable to prevent end-stage organ damage associated with diabetes.[97,98] However, not all investigators were able to reproduce the inhibitory effects of HRP either *in vivo* or *in vitro*.[97,99]

In diabetic subjects, prorenin to active renin ratio is very high. This is supported from studies where the excessive non-proteolytic activation of (P)RR-bound prorenin has a major role in diabetic nephropathy and retinopathy, and cardiac fibrosis.[86,88,100–102] As a result of high prorenin level in these diseases, the HRP blocked prorenin binding to the (P)RR (enzymatic activation) and non-proteolytic activation of prorenin by (P)RR. This suggests overexpression of (P)RRs and a predominant role of RAS in these disease conditions.[88] On the other hand, high prorenin noted in pregnancy[103] or patients treated with drugs targeting RAS[104] did not result in end-organ damage. Similarly, in transgenic animals, where circulating prorenin was 200 times more than renin failed to cause cardiac or kidney fibrosis but were hypertensive, and the increase in blood pressure could be antagonized by Ang-converting enzyme inhibitors.[105,106]

The discrepancy in the effect of HRP, and the absence of an end-organ damage in pregnancy and transgenic animals are probably because of: (a) decreased expression of cathepsin enzyme in kidneys of diabetic rats,[107] (b) absence of non-proteolytic activation of prorenin because of downregulation of (P)RR via activation of the transcription factor promyelocytic zinc-finger pathway,[108] (c) subcellular localization and intracellular processing of (P)RRs and their association with v-H^+-ATPase,[107,109] (d) the varying affinity of the molecular forms of (P)RRs for (pro)renin[109] and (e) the difference in distribution of full-length receptor to soluble receptor[107] in a disease condition. A recent report suggests that angiotensinogen is cleaved differently by free renin and renin bound to (P)RR.[110] Enzymatic activity of (P)RR-bound renin is higher than free renin. Oxidized angiotensinogen more effectively releases Ang in the presence of renin bound to (P)RR.[110]

The (P)RR is a fusion of two functionally distinct domains, a vertebrate-specific extracellular domain that is implicated in (pro)-renin binding and signaling, and the evolutionarily conserved or ancient transmembrane domain and a small intracellular tail, which are essential for cell survival.[27] The latter part (ancient segment) of the receptor (called M8.9), which is identical to the ATP6ap2 protein in sequence, co-precipitates with the v-H^+-ATPase providing evidence for an association between the (P)RR and the enzyme.[111,112] As a result of this, the (P)RR gene is named *ATP6ap2*, which codes for the (P)RR and the fragment M8.9.[109] A functional link between the receptor and v-H^+-ATPase has been demonstrated in renal intercalated cells in the collecting ducts.[113] These investigators showed that (pro)renin can increase, whereas bafilomycin can reduce v-H^+-ATPase activity via, respectively, activation or inhibition of the (P)RR.[113]

The v-H^+-ATPase is a multisubunit complex molecule that is organized into the V_1 (eight subunits) and V_O (six subunits) sectors and two accessory subunits (Ac45 and (P)RR/ATP6ap2) localized mainly within intracellular compartments and to a minor part at the plasma membrane.[91,108,112] The (P)RR/ATP6AP2 senses the acidity levels of the intracellular compartments and accordingly regulates v-H^+-ATPase activity.[112] The disruption of pH homeostasis in v-H^+-ATPase mutants showed lethality in various organisms.[114] In murine cardiomyocytes, ablation of *ATP6ap2* selectively suppressed protein expression of the V_O subunits of v-H^+-ATPase resulting in loss of function for v-H^+-ATPase leading to de-acidification of intracellular vesicles.[112] The (P)RR is essential along functional link with the v-H^+-ATPase for cell survival and in early organ development.[87,111,115] In rodents, the receptor expression is ubiquitous and occurs early in development,[116] and in humans, (P)RR/ATP6ap2 mutation results in mental retardation and epilepsy[117], and the lack of it may impair neurotransmission.[118] Thus, the (P)RR is slated to act in two ways: (a) as the receptor for renin and prorenin generates Ang-II and triggers the activation of the MAPKs extracellular signal-regulated kinase 1/2 signaling pathways resulting in hypertension, cardiac fibrosis and glomerulosclerosis, and (b) as a protein associated with the v-H^+-ATPase, exerts v-H^+-ATPase-associated functions essential to Wnt signaling.

THE CROSS-TALK: (P)RRS AND WNT/FZ SIGNALING SYSTEM

Studies of G protein-coupled receptors and protein tyrosine kinases have shown that endocytic transport and endosomal acidification are important events in regulating signal transduction and mediating the formation of specialized signaling complexes.[119] The endosomal pH affects ligand-receptor affinities and alters conformational changes to influence the downstream effector signal transduction.[120] Recent studies of Wnt/Fz signaling suggest that the binding of Wnt to Fz receptors and the activation of Dvl are achieved by a local change in pH. Acidification in intracellular vesicles and plasma membranes for Wnt–Fz binding and subsequent signaling is performed largely by v-H^+-ATPase.[30] On binding of Wnt to Fz, the Wnt/Fz-LRP6-(P)RR-v-H^+-ATPase complex is endocytosed, wherein the v-H^+-ATPase creates an acidic environment for LRP6 phosphorylation/activation, which is needed to activate Wnt/Fz signaling, followed by cytosolic β-catenin activation. Apicularen and bafilomycin, specific inhibitors of v-H^+-ATPase, both inhibited the activation of LRP6 but did not affect T-cell factor promoter activation by β-catenin.[121] These findings suggest that v-H^+-ATPase inhibition interferes with upstream events such as binding to ligand-activated Fz and recruitment of Dvl at the plasma membrane level.[122] A local change in pH is also required for non-canonical Wnt signaling because the organization of the actin cytoskeleton is strongly pH-dependent. For non-canonical signaling, both v-H^+-ATPase/(P)RR and Nhe2 (a Na^+/H^+ exchanger) are implicated in Dvl recruitment to the membrane.[29,61]

A number of recent studies suggest the regulatory or modulatory role of the (P)RR associated with the v-H^+-ATPase in both canonical (Figure 1) and non-canonical (Figure 2) Wnt signaling.[28–31,118] In *Xenopus* embryos and cultured cells, Cruciat *et al.*[28] demonstrated that the (P)RR is a prerequisite for Wnt/β-catenin signaling, where the receptor functions downstream of Wnts and upstream of β-catenin. The (P)RR places its action at the level of Fz or upstream of its co-receptor LRP6 and thus described as a specific adaptor between Fz, coreceptor LRP6, and v-H^+-ATPase complex.[28,29,118] Furthermore, the investigators observed that the (P)RR did not directly transduce a cytoplasmic signal in Wnt pathways; rather it functioned in a renin-independent manner.[28] These findings were a little time later confirmed in a *Drosophila* model that a (P)RR homolog binds to Fz receptors in both the Wnt/β-catenin and the Wnt/JNK/PCP pathways.[29,30] A loss or depletion of the (P)RR led to a reduction of Wg (wingless) target gene expression. These studies support the argument involving totally renin-independent mechanisms in (P)RR/v-H^+-ATPase-induced modulation of Wnt signaling as *Drosophila* does not have renin and *Xenopus* embryos do not express renin at the early phase of development. Several investigators while evaluating the efficacy of HRP in a variety of experimental models on (P)RR-bound (pro)renin-induced cardiac hypertrophy, hypertension, albuminuria, renal damage and nephrosclerosis have failed to demonstrate its beneficial effects.[123–125] These findings suggest that (P)RR is multifunctional, more complex than what has previously been thought, and may possibly involve additional endogenous agonist(s) other than prorenin.

In collecting duct Madin–Darby canine kidney cells, Advani *et al.*[113] showed that (pro)renin can increase v-H^+-ATPase activity via activation of (P)RR. Furthermore, they reported that MAPKs extracellular signal-regulated kinase 1/2 phosphorylation induced by (pro)renin was markedly inhibited by bafilomycin, a selective inhibitor of v-H^+-ATPase. The v-H^+-ATPase and its associated protein, (P)RR, are predominantly expressed at the apical surface of the collecting duct where it functions to expel protons into the tubular lumen, thereby contributing to urinary acidification.[126] The findings that the collecting duct is a key site of local RAS activity, where renin is produced in abundance by collecting duct principal cells[127] suggest an Ang-II-dependent activation of the collecting duct v-H^+-ATPase and modulation of urinary acidification.[128,129] In agreement with these results, Advani *et al.*[113] concluded that the (P)RR has a primary role in distal nephron proton transport, which at least in part, an Ang-II-dependent phenomenon.

The subcellular localization of the (P)RR adds new dimension to the receptor activation of v-H^+-ATPase in the Wnt receptor complex

signaling. A major amount of the receptor is located on intracellular vesicles, whereas only a fraction is found on the plasma membrane.[91,108] In contrast, a predominance of (P)RR expression was noted at the luminal surface of rat kidney collecting duct intercalated cells.[102] The full-length (P)RR, after trafficking to the Golgi, is cleaved by protease furin to liberate a 28 kDa fragment called soluble (P)RR and the 8.9 kDa fragment.[109] Soluble (P)RR is found in both rat and human plasma[109] and human urine.[107] But, a majority of soluble (P)RRs may be found in the extracellular space and a minor fraction in plasma because of digestion by plasma protease.[107] Like the full-length (P)RR, soluble (P)RR can bind (pro)renin but it is not clear whether it competes with the membrane (P)RR.[109,118] These factors might influence the function of (P)RR in Wnt signaling.

The essential role of the (P)RR as a v-H^+-ATPase accessory subunit in the Wnt signaling pathway in renin-independent basic cellular functions is supported by the following reports: (a) mutation of (P)RR affected the development of zebrafish and *Xenopus* embryos resulting in small head, shortened tail, defect in melanocyte and eye pigmentation, defect of neural patterning and death of the embryo,[28,115,118] (b) mouse embryonic stem cells deficient for *ATP6ap2* resulted in preimplantation lethality of the embryo,[111] (c) human (P)RR/ATP6ap2 mutation was associated with mental retardation and epilepsy[117] and (d) genetic ablation of *ATP6ap2* in murine cardiomyocytes created a loss of function model for v-H^+-ATPase and mice died within 3 weeks of birth.[112] As noted in early sections, Wnt signaling is essential for numerous processes in embryonic development[1,2] and probably is the reason for failure to generate (P)RR knockout mice.[118] These observations suggest that (P)RR has functions essential for survival and proliferation that are likely unrelated to the RAS[87] and as a cofactor of v-H^+-ATPase, is essential in Wnt/Fz complex signaling. A number of investigators have observed the crucial role played by the (P)RR and Wnt signaling in normal development of cardiovascular and renal systems. Aberrant activation of these pathways has led to heart fibrosis, renal fibrosis, glomerulosclerosis, cystic disease and diabetic nephropathy.[6,112,130–133] Overexpression of the (P)RR in the rat and mouse resulted in hypertension and fibrotic effects.[91,111,130] This raises several questions. How does the binding of prorenin to the (P)RR affect the function of v-H^+-ATPase and contribute to generation of Ang (hypertension) and trigger MAPK signaling pathways (glomerulosclerosis, heart fibrosis) in a renin-independent manner? Furthermore, it will be important to understand the significant overlap between the two functions of the (P)RR, a receptor for (pro)renin identified as a component of the RAS, and a cofactor via its interaction with the v-H^+-ATPase in Wnt signaling.

The Wnt proteins are growth factors and are involved in cardiac differentiation and development, and angiogenesis.[3,4,41,134] The gene products of ATP6ap2 are essential for cardiomycocyte survival via regulating v-H^+-ATPase function as demonstrated by Kinouchi *et al.*[112] in mice. In this study, the investigators observed that the genetic ablation of *ATP6ap2* resulted in heart failure and the mice died within 3 weeks of birth. This was the result of v-H^+-ATPase dysfunction,[112] which probably impaired Wnt signaling since Wnt proteins control every stage of cardiac development. The contribution of defective Wnt/Fz signaling to congenital cardiac malformation has been described in several species.[3] Along with others,[10,77,82,135–138] we have extensively reviewed the generally accepted fact that the hypertrophic responses of the heart involve the re-expression of the fetal gene program, suggesting that either Wnt signaling is reactivated, or the abnormal activation of Wnt signaling occurs. On binding of Wnt to Fz-LRP5/6 complex, the Dvl protein is activated, resulting in the inhibition of GSK-3β activity involving destabilization of the β-catenin degradation complex. GSK-3β, a central component of the Wnt/Fz signal transduction pathways and a negative regulator of cardiomyocyte hypertrophy,[136] is a cytoplasmic antihypertrophic protein involved in nuclear export of NFAT and GATA on dephosphorylation,[137] which are directly implicated in driving cardiac hypertrophic gene transcription.[9] Additionally, the Wnt-mediated inhibition of GSK-3β increases the amount of cytoplasmic β-catenin. This, in turn, results in β-catenin-mediated hypertrophic gene expression with T-cell factor/lymphoid enhancer factor, which is required for stress-induced cardiac hypertrophy[138] (Figure 1).

Apart from the role of Wnt/Fz in the regulation of cardiac hypertrophy, a number of studies have also implicated the Wnt signaling pathway in renal fibrosis, glomerulosclerosis and renal dysfunction.[131,133] Impaired Wnt/PCP signaling is associated with abnormal kidney repair.[31] As noted above, (P)RR blockade inhibited the development and progression of glomerulosclerosis in Ang-II type 1a receptor-deficient diabetic mice, while RAS inhibitors only slowed the progression of diabetic nephropathy.[88] This suggests RAS-independent mechanisms to the development of nephropathy[88] and the involvement of (P)RRs in the Wnt signaling pathways. Such studies have highlighted the importance of regulating upstream target molecules such as Dvl and GSK-3β rather than the cytoplasmic β-catenin, the transcriptional activator. In the absence of studies describing mutations in Wnt/Fz signaling,[9] it is prudent to suggest that interventions at the membrane level, upstream of β-catenin production, may have a significant role in modulating cardiovascular and renal diseases. The studies of Buechling *et al.*[29] and Hermle *et al.*[30] strongly suggest that the (P)RR has a supplementary role in regulating canonical as well as non-canonical Wnt signaling at the plasma membrane level. On the basis of these reports, we suggest that the (P)RR-mediated signals for induction of cardiovascular abnormalities such as hypertension, cardiac hypertrophy and heart failure may route through non-canonical Wnt/JNK and Wnt/Ca^{2+} signaling pathways (Figure 2) in addition to canonical Wnt/β-catenin signaling pathways (Figure 1).

Unlike Fz or Ang-II type 1 receptors, neither the (P)RR nor the v-H^+-ATPase is a signaling receptor,[139] and are not coupled to G proteins or any accessory enzymes and have no intrinsic kinase activity.[25] Thus, the ligand-bound (P)RR activating v-H^+-ATPase must activate another receptor[140] with a signaling property analogous to its own, such as Fz receptor. Of note, Fz receptors also respond to non-Wnt ligands, for example, the best studied Norrin.[141] A direct relationship between Fz and (P)RRs seems likely because each of their signaling pathways are implicated in these diseases. It is intriguing to speculate that the (P)RR could have a detrimental role in cardiac fibrosis and glomerulosclerosis, possibly through co-activation of Wnt signaling pathways at the membrane level. These observations demonstrating the involvement of (P)RRs in v-H^+-ATPase function and in Wnt signaling pathways have extended the functional spectrum of multifunctional (P)RRs from the end-organ damage in hypertension and in diabetic complications to the embryonic development, tissue repair and degenerative diseases. The puzzling question is: how is Wnt signaling differentially initiated and regulated in a specific manner by the (P)RR–v-H^+-ATPase at the plasma membrane level? The underlying molecular mechanisms are unclear.

CONCLUDING REMARKS

Wnt/Fz and (P)RR signaling pathways are essential for cell survival and are an integral part of early embryonic development. However, aberrant activation of the (P)RR and Wnt/Fz signaling is implicated in cardiovascular diseases, renal fibrosis, glomerulosclerosis and

proteinuria.[10,26] Recently, a number of investigators have suggested a modulatory or regulatory role of the (P)RR in the Wnt/Fz signaling pathway at the level of Fz receptor, consistent with it being a transmembrane receptor.[28–30] The (P)RR is an accessory subunit of the v-H^+-ATPase as it binds to several v-H^+-ATPase subunits. A functional link between the (P)RR and v-H^+-ATPase has been identified, wherein the blockade of v-H^+-ATPase has attenuated the increase in extracellular signal-regulated kinase 1/2 phosphorylation induced by (pro)renin.[113] The v-H^+-ATPase serves as a pH sensor and is required for Wnt–Fz binding and subsequent signaling in intracellular vesicles and at the plasma membrane. Based on results from a number of elegant studies done in *Xenopus*[28] and *Drosophila*,[29,30] it can be postulated that the (P)RR modulates Wnt/Fz signal transduction through interaction with Wnt/Fz-LRP6, Wnt/JNK/PCP and Wnt/Ca^{2+} at or near the cell membrane. Thus, we reason that very high and persistent levels of prorenin (observed in cardiovascular and diabetes-related renal and cardiac end-organ damage) can increase v-H^+-ATPase activity via activation of the (P)RR in Wnt/Fz signaling. At present, it is not apparent whether the functions of (P)RR and v-H^+-ATPase in Wnt signaling pathways are renin-independent as suggested by Cruciat *et al.*[28] although the physiology and pathophysiology of (P)RR signaling remains incomplete. This could be confirmed by studies using either tissue-specific (P)RR knockout animals or selective (P)RR antagonists. However, no (P)$RR^{-/-}$ mice could be generated.

We[26] have recently reviewed studies demonstrating the crucial role of the (P)RR in the pathophysiology of cardiovascular and renal end-organ damage and the receptor as a therapeutic target over current drugs, as none of which effectively treat these serious conditions. This raises several questions because of identification of two functions for the (P)RR, (pro)renin binding to the (P)RR (Ang-II generation and triggering several MAPK signaling pathways) and activation of v-H^+-ATPase in the Wnt/Fz receptor complex signaling pathways. Are these two functions interconnected, or are they independent of each other? Are each of these functions mediated by different subtypes of (P)RRs? Can a (P)RR antagonist block both the Ang-II-dependent and -independent effects of (pro)renin, and also inhibit (P)RR associated v-H^+-ATPase function and their influence on Wnt/Fz signaling? Currently, there are no pharmacologic tools available to specifically inhibit either the (P)RR or the Wnt/Fz signaling pathway. This makes the (P)RR a therapeutic target for cardiovascular and renal pathologies and degenerative diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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