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Beyond Cell Adhesion: the role of Armadillo proteins in the heart

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Abstract

Plakoglobin (PG, γ -Catenin, JUP), a member of the armadillo protein family and close homolog of β -catenin, functions to link cell surface cadherin molecules with the cytoskeleton. PG is the only junctional component found in both desmosomes and adherens junctions and thus plays a critical role in the regulation of cell-cell adhesion. Similar to β -catenin, PG is able to interact with components of the Wnt signaling pathway and directly affect gene expression by binding with LEF/TCF transcription factors. In addition, it has been proposed that PG functions primarily as a competitive inhibitor of β -catenin transcriptional activity by sequestering LEF/TCF. Compared to β -catenin, the contribution of PG as a transcriptional regulator in either physiological or pathological conditions is poorly understood. There is increasing clinical interest in PG as both a structural protein as well as a signaling molecule as mutations have been identified in the human PG gene that cause Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) and cutaneous syndromes. This review will discuss the connection between altered cell adhesion and gene expression and its contribution to disease pathogenesis.

Keywords

plakoglobin; β -catenin; cadherin; adherens junction; desmosome; Wnt signaling; arrhythmogenic right ventricular cardiomyopathy (ARVC)

Introduction

Plakoglobin (PG, γ -catenin, JUP) was initially described as a component of multiple intercellular mechanical junctions [1]. It was later found to be homologous to the *Drosophila* segment polarity gene; *armadillo* (arm) [2, 3]. Shortly following the discovery of PG, another arm family member was recognized as β -catenin (CTNNB1). Initially, it was proposed that PG and β -catenin might be the same molecule as they were so closely related, however further studies soon revealed two distinct proteins [4, 5]. Over the years β -catenin has been extensively studied (~14,000 articles PubMed), compared with the enigmatic PG molecule (~1,300 articles). This discrepancy is due to the well-established signaling capacity of β -catenin as part of the Wnt pathway and as such its importance in developmental biology, tissue regeneration and disease pathogenesis. In contrast, current opinions regarding the physiological significance of PG as a transcriptional regulator remain controversial.

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Armadillo protein family members have a central “armadillo” domain containing 13 imperfect repeats of 42 amino acids which is flanked by distinct amino (N-) and carboxy (C-) terminal domains [6]. The positively charged groove of the arm domain is organized as α -helixes and mediates numerous protein-protein interactions discussed below. The central arm domain of PG and β -catenin has 83% amino acid similarity while the N- and C-terminal tails share 57% and 15% similarity, respectively (Fig. 1) [7]. The relatively low homology between the terminal tails likely contributes to disparate binding partners and functional activity of these otherwise closely related armadillo family members. This review will focus on PG and β -catenin as structural proteins and their roles in signal transduction in the heart. We will discuss recent evidence supporting an unexpected role for PG as a negative regulator of Wnt/ β -catenin signaling in cardiac progenitor cells and its relevance to the unique fibro-fatty pathology observed in ARVC patients.

Functional overlap and distinct roles for PG and β -catenin

Armadillo protein function in cell adhesion

Desmosomes and adherens junctions act as mechanical cell-cell adhesion junctions maintaining the structural integrity of the tissue. Adherens junctions are comprised of classical single membrane pass cadherins that bind to like cadherins on neighboring cells, forming a cell adhesive zipper [8]. PG and β -catenin bind to the cytoplasmic tail of cadherins in a mutually exclusive manner. E-cadherin binds PG directly between arm repeats 4–5 [4, 9], while N-cadherin binds to repeats 7–8 [10]. PG and β -catenin mediate linkage of cadherins to the actin cytoskeleton via α -catenin, although not necessarily through a direct physical link [11]. Arm repeat 1 has been shown to be essential for PG interaction with α -catenin [10, 12–14] (Fig. 1). Desmosomes also provide cell-cell adhesion, but do so through intracellular connection to the intermediate filament system. PG plays a critical role in linking the desmosomal cadherins, desmoglein (DSG) and desmocollin (DSC), to the cytoskeleton via desmoplakin (DP). Plakophilin, another member of the armadillo protein family, like PG binds to the cytoplasmic domain of DSG and DSC and serves to link the transmembrane proteins to the cytoskeleton [15, 16]. Similar to adherens junctional components, desmosomal cadherins bind the central arm repeats of PG [17–26] (Fig. 1). PG and β -catenin bind with similar affinities to E-cadherin, whereas PG binds to DSG-1 stronger than does β -catenin [27]. Moreover, β -catenin fails to recruit DP and anchor desmosomal cadherins to intermediate filaments in PG-null cells [28, 29]. Therefore, β -catenin cannot functionally replace PG in desmosomal junctions. This is an important distinction as PG mutations are associated with a cardiocutaneous syndrome that is likely explained by altered cell adhesion in the heart and skin of those patients. This topic will be discussed later in relation to the genetic disease arrhythmogenic right ventricular cardiomyopathy (ARVC).

Proper mechanical and electrical coupling of cardiomyocytes is crucial for normal propagation of the electrical impulse throughout the working myocardium. Junctional proteins concentrated at the termini of cardiomyocytes are responsible for the integration of structural information and cell-cell communication. The end-to-end connection between cardiomyocytes called the intercalated disc (ICD) consists of three main junctional complexes: adherens junctions, desmosomes, and gap junctions. The gap junction provides intercellular communication via small molecules and ions that pass through a channel generated by a family of proteins called connexins. Gap junctions allow for electrical coupling of cardiomyocytes ensuring coordinated muscle contraction required for proper heart function. There is a growing appreciation for the integrated nature of the junctional complexes at the ICD and how aberrant cell-cell coupling mediated through these junctions can lead to cardiomyopathy and an increased risk of arrhythmias.

Armadillo protein function in signal transduction

The Wnt signal transduction pathway controls a variety of biological phenomena throughout development and adult life of all animals [30]. Aberrant Wnt signaling underlies a wide range of pathologies in humans. β -catenin is a well-established downstream effector of the Wnt signaling cascade. In contrast, whether PG is a positive or negative regulator of Wnt signaling remains a matter of discussion. PG and β -catenin co-exist in a tightly regulated cytoplasmic pool between membrane associated forms and nuclear localization (Fig. 2). Cytosolic pools of β -catenin/PG are managed by canonical Wnt signaling and ubiquitin-proteasome-dependent degradation. The targeting of β -catenin/PG degradation is achieved through the phosphorylation of N-terminal serine residues by a multi-protein “destruction complex” containing glycogen synthase kinase 3 β (GSK3 β) and scaffold proteins adenomatous polyposis coli (APC) and axin [31–33]. The GSK3 β -dependent phosphoserine motif of PG is recognized by the ubiquitin ligase β -TrCP that targets PG for degradation via the 26S proteasome [34]. Degradation of β -catenin/PG by the destruction complex is regulated by the binding of Wnt to its receptor, frizzled (FRZ) and transactivation of scaffold protein, disheveled (DSH) [35]. Activated DSH recruits GSK-binding protein (GBP/FRAT) and protein phosphatase 2C leading to the disassembly of the destruction complex and accumulation of β -catenin/PG in the nucleus [36–42]. Despite the fact that these proteins interact with the same degradation machinery, PG protein stability does not appear to be as tightly controlled as β -catenin. Mutations in the GSK3 β phosphorylation domain of β -catenin (e.g. S37A) cause a dramatic increase in cytoplasmic levels of the protein and its signaling activity. However, the analogous N-terminal mutation in PG (S28A) does not confer extra stability or activity compared to the wild-type protein [43]. This likely explains the lack of activating mutations identified in PG whereas mutations that stabilize β -catenin are often observed in human tumors [44].

β -catenin and PG lack DNA binding domains and must bind to HMG box-containing, DNA binding proteins in order to regulate transcription. Specifically, they interact with members of the T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF) family of transcription factors [45–48]. Similar to β -catenin, PG contains a transcriptional transactivation domain in its C-terminus [45, 47], however there is less than 15% amino acid similarity between these domains. The central arm domain of both PG and β -catenin interact with Tcf-4, and the N- and C- terminal tails can also bind the arm domains and determine the specificity and affinity of each protein for the co-factors [7]. Studies have suggested that PG-TCF/LEF cannot form a strong ternary complex with DNA, however transfection of PG mutants lacking the N- and C-terminal tails induced a PG-TCF/LEF-DNA transcriptionally active complex [48]. Moreover, PG and β -catenin bind mutually exclusive sites on Tcf-4 and ternary complexes of β -catenin/PG/Tcf-4 can be found, indicating simultaneous binding of both armadillo proteins to Tcf-4. Experiments performed with Tcf-4 mutants with altered PG binding sites displayed increased transcriptional activity of Tcf-4, suggesting an antagonistic function of PG towards Wnt/ β -catenin signaling activity [49]. The lack of amino acid similarity between the C-terminus of PG and β -catenin might explain why PG is less transcriptionally active compared to β -catenin.

Several groups have shown that PG has limited ability to activate TCF/LEF-dependent transcriptional activity [43, 48, 50]. These earlier studies utilized overexpression systems with cell lines containing endogenous PG and β -catenin, which could interfere with the regulation of target genes. More recently PG was shown to be insufficient to activate TCF/LEF-dependent transcription in β -catenin-null F9 cells following Wnt3a stimulation [51]. On the other hand, in a mesothelioma cell line (NCI-H28) devoid of endogenous β -catenin, PG co-transfection with TCF4 or LEF1 caused an increase in transcriptional activity [52]. Moreover, knockdown of endogenous PG resulted in a modest decrease in TCF/LEF

reporter activity. Collectively, these studies suggest the signaling capabilities of PG are considerably weaker than that of β -catenin, but may be present nonetheless.

Recent evidence supports a direct role for PG in the regulation of gene expression. Using a keratinocyte differentiation model, it was shown that PG suppresses c-Myc expression in a Lef-1-dependent manner [53]. The PG-mediated suppression was similar in wild-type and β -catenin-null cells demonstrating PG could regulate gene expression independent of β -catenin. Chromatin immunoprecipitation (ChIP) analysis indicated that PG and Lef-1 associated with the c-Myc promoter in keratinocytes undergoing growth arrest. The same group performed a genome-wide promoter screen and identified over 5,000 PG target promoters by ChIP followed by promoter array analysis (ChIP-chip) in differentiating skin keratinocytes (Müller, EJ unpublished data). PG was found to target both Wnt pathway components and Wnt target genes. Surprisingly, the same approach identified only 2,000 promoters for β -catenin with a 38% overlap with PG target genes. With respect to Wnt target genes, PG was found to target and suppress c-Myc and cyclinD1 whereas it activated Dsg3 in a Lef-1-dependent manner. Thus, PG can directly regulate gene transcription in primary keratinocytes suggesting its function is cell-type specific and likely depends on the differentiation state of the cell and the relative expression levels of a variety of interacting proteins including cadherins.

Embryonic requirement for PG and β -catenin

Consistent with their essential roles in tissue morphogenesis germline deletion of either PG or β -catenin result in embryonic lethality. PG-null animals suffer from cardiac rupture and begin to die at embryonic day (E)10.5 [54, 55]. Depending on the genetic background some animals survive longer and exhibit severe skin blistering and subcorneal acantholysis. The cell adhesion defects can be explained by the disruption in desmosomal integrity. The phenotypic changes in the heart and skin of PG-null mice are likely explained by the amount of mechanical stress each tissue undergoes. In comparison, β -catenin knockout mice die earlier at E7.5 due to defects in the ectoderm [56]. Although β -catenin is ubiquitously expressed throughout the embryo only the ectoderm layer exhibits a cell adhesion defect whereas extraembryonic structures are unaffected. To bypass the early lethality, β -catenin was depleted in specific cell lineages demonstrating the importance of Wnt/ β -catenin signaling in numerous developmental processes including cardiac development [57]. Thus, loss-of-function (LOF) studies indicate impaired cell adhesion as the primary defect in the PG-null embryos whereas β -catenin is essential for early specification of the body plan. In conclusion, PG cannot compensate for the absence of β -catenin in developmental signaling processes and conversely β -catenin fails to functionally substitute for PG in desmosomal junctions.

β -catenin signaling in the stressed myocardium

Terminally differentiated cardiomyocytes do not require canonical Wnt/ β -catenin mediated signaling at least under normal conditions. Cardiac-specific deletion of β -catenin in the nonstressed adult myocardium demonstrated that β -catenin is dispensable for normal cardiac function [58]. The lack of phenotype is presumably due to the upregulation of PG in the β -catenin conditional knockout (CKO) heart thus functionally replacing β -catenin in adherens junctions. In comparison, in β -cat ^{Δ ex3} mice a stabilized form of β -catenin accumulates at the ICD and causes cardiomyopathy suggesting the stoichiometry of the armadillo proteins is important for proper function of the mechanical junctions in the heart [59]. In support of this idea, cardiomyopathy is also observed in mice overexpressing PG in the heart [60]. Interestingly, there is no nuclear accumulation of β -catenin or increase in transcription of β -catenin target genes in the cardiac-specific β -cat ^{Δ ex3} mice under normal physiological

conditions [59]. However, as discussed below, β -catenin-mediated signaling plays a critical role in cardiac remodeling in response to stress or injury.

Upon pathological stress, the heart reactivates several signaling pathways involved in cardiac development including Wnt/ β -catenin signaling [57]. The β -catenin mutant mice have been subjected to various stress or injury protocols in order to investigate the requirement for β -catenin in cardiac remodeling (Table I). Following transverse aortic constriction (TAC) to induce pressure overload, β -catenin CKO mice exhibit reduced Wnt target gene expression and are unable to undergo a pathological hypertrophy response [61]. Moreover, even α MHC-Cre; β -cat^{fl/+} mice (i.e. β -cat haploinsufficiency in cardiomyocytes) have an attenuated cardiac hypertrophy response following TAC demonstrating the importance of β -catenin in cardiac remodeling [62]. Furthermore, cardiac-specific expression of a Lef1 dominant negative mutant failed to display hypertrophy after myocardial stress (i.e. TAC) consistent with a requirement for a β -cat/Lef transcription complex [61]. Although, β -catenin inhibition appears to be cardio-protective following injury, the mechanism behind increased cardiac function and reduced infarct size is controversial. Some investigators suggest that loss of β -catenin results in enhancement of cardiac progenitor cell differentiation post injury as opposed to inhibition of hypertrophy [63]. Epicardial cells have also been implicated in β -catenin signaling following cardiac injury. Following acute ischemia, Wnt1 expression is upregulated in epicardial cells and cardiac fibroblasts [64]. The epicardial cells are stimulated by Wnt/ β -catenin signaling to undergo epithelial to mesenchymal transition (EMT) to promote cardiac repair. Inhibition of β -catenin in epicardial cells results in decreased epicardial expansion, reduced collagen replacement, and reduced cardiac function following ischemia reperfusion injury [65]. Taken together, these results indicate the importance of Wnt/ β -catenin signaling in the cardiac remodeling process associated with heart disease. Moreover, a distinct phenotypic change in β -catenin mutant mice following cardiac stress implies that PG is unable to functionally compensate for β -catenin-mediated signaling in the stressed adult heart. It remains to be determined whether PG has a role in stress-induced pathological hypertrophy.

ARVC a disease of the desmosome

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heritable condition characterized by distinct pathology resulting from dramatic cardiomyocyte cell death and subsequent fibro-fatty replacement of the muscle [66]. ARVC is one of the most common inherited cardiomyopathies and a prevalent cause of sudden cardiac death (SCD) in young adults, reportedly contributing to up to 10% of SCD in individuals less than 65 years old [67]. While originally termed ARVC, recent evidence has shown that both right and left ventricles are often affected in these patients. As such, a descriptive change to Arrhythmogenic Cardiomyopathy has been suggested to better reflect the pathology [68, 69]. Almost half of ARVC patients have a mutation in one of the genes encoding desmosomal proteins expressed in the heart: PG (JUP), plakophilin2 (PKP2), desmoplakin (DSP), desmocollin2 (DSC2), and desmoglein2 (DSG2). Thus ARVC is considered a disease of the desmosome [70]. The majority of desmosomal mutations have been identified in the *PKP2* gene [71, 72]. Most of the desmosomal mutations are dominant although recessive mutations have also been identified in ARVC patients. Patients suffering from ARVC, regardless of their desmosomal gene mutation, have diminished PG localization at the ICD [73] suggesting an important role in disease progression (to be discussed later). On the basis of this finding it has been proposed that PG loss from the ICD may serve as a diagnostic marker of the disease [73].

A hallmark of ARVC is incomplete penetrance and variable expressivity of the disease phenotype making it difficult for clinicians to advise patients of their risk of SCD. Adding

further to the genetic complexity ARVC patients were recently identified with more than one mutation in the same or different desmosomal gene, suggesting that ARVC might require multiple genetic hits in the cell adhesion complex to elicit a cardiac phenotype [74–76]. Additional genetic analysis is required to identify modifier genes that might contribute to the variable expressivity of the ARVC phenotype.

Mutations in the human *JUP* gene

The first genetic mutation discovered leading to ARVC was a homozygous 2 base pair deletion (2057del2) in the *JUP* gene causing a frame-shift and premature truncation of the PG protein (G680fsX690) [77] (Fig. 3). The disease was originally identified in patients on the Greek island of Naxos hence it is referred to as “Naxos disease” [78]. In addition to heart problems, Naxos patients exhibit palmoplantar keratoderma and woolly hair [79]. As described earlier, desmosomes form essential mechanical junctions important for maintaining the structural integrity of the heart. The truncated form of PG (i.e. Naxos) when transfected into HEK293 cells displays nonjunctional localization leading to decreased cell-cell adhesion supporting the hypothesis that defective mechanical coupling between cardiomyocytes is involved in the etiology of ARVC [80]. Perturbations in desmosome structure affect tissue systems that undergo significant mechanical stress, implicating the heart and skin. Cutaneous phenotypes have also been observed in a subset of ARVC patients carrying mutations in different desmosomal genes. A dominant mutation in the PG gene was recently discovered causing ARVC with no cutaneous abnormalities (Fig. 3). The mutation (S39_K40insS) results in the insertion of an extra serine residue at position 39 of the N-terminus of PG that causes a decrease in PG localization at the ICD [81]. Transfection of the mutant PG into HEK293 cells demonstrated mislocalization of the protein away from the plasma membrane to the cytoplasm. The molecular explanation for the lack of cutaneous abnormalities in patients carrying the PG S39_K40insS mutation remains unclear. A recent novel mutation in the *JUP* gene (Q539X) results in severe congenital skin fragility with generalized epidermolysis and massive trans-cutaneous fluid loss [82] (Fig. 3). The child homozygous for this mutation died 12 days after birth. Ultrasound did not detect any cardiac problems, however no autopsy was performed on this patient so it is unknown whether the cytoarchitecture of the heart was normal. Five children from different families were recently identified carrying homozygous nonsense (S24X) or missense (468G>A) mutations in PG that elicit skin fragility, palmoplantar keratoderma and woolly hair [83] (Fig. 3). Interestingly, echocardiography has not yet detected any cardiac abnormalities in these adolescent patients. Keratinocyte-specific PG knockout mice recapitulate the severe epidermal defects observed in patients carrying *JUP* mutations [84].

Murine models of PG dysfunction

Over the past several years, researchers have begun to investigate the molecular mechanisms responsible for ARVC by generating conditional knockout mice where the desmosomal gene of interest is depleted specifically in the heart. Additionally, transgenic mice expressing mutant desmosomal genes have also proved valuable in understanding the role of specific human mutations in ARVC [85]. Although these are the early days in this research field, these animal models have provided important insight into the disease pathogenesis. For this review, we will focus on genetically engineered mouse models where PG levels are altered in the heart (Table I).

Using a conditional knockout approach, our group recently reported that depletion of PG in the adult murine heart causes altered desmosome ultrastructure and decreased cardiac function as determined by echocardiography [86]. The cardiac pathology in the PG mutant mice recapitulates many features of ARVC including progressive loss of myocytes, extensive inflammatory infiltration, and replacement fibrosis. However, we did not observe

the presence of adipocytes in the PG CKO model. As discussed below, Wnt/ β -catenin signaling is known to suppress adipogenesis [87]. Indeed, we observe an increase in β -catenin protein levels, interaction with TCF-4, and Wnt/ β -catenin target gene expression in the absence of PG, providing a possible explanation for the lack of adipocytes in the PG CKO model. Additionally, the PG mutant mice were not susceptible to arrhythmias and survived longer than expected based on the human data [86]. The lack of an arrhythmogenic phenotype may be explained by the fact that PG was deleted in the mature heart in our model whereas another group deleted PG earlier in the perinatal period and observed rapid development of the ARVC phenotype including arrhythmia [88]. Moreover, pathological stress was associated with increase in TGF β 1 signaling in the PG-depleted hearts [88]. To further understand the pathogenesis of the human disease the next generation murine models will utilize knockin alleles of the specific *JUP* mutations identified in ARVC patients.

PG signaling in ARVC

A hallmark of ARVC is the loss of cardiomyocytes that are replaced with fibroblasts and adipocytes. The molecular mechanism(s) underlying this unique fibro-fatty pathology highlight the dual function of the armadillo proteins as both structural components of mechanical junctions as well as transcriptional regulators (Fig. 4). The canonical Wnt/ β -catenin signaling is an important regulator of myogenesis versus adipogenesis [87]. Activation of Wnt/ β -catenin signaling enhances myogenesis but inhibits adipogenic transcription factors C/EBP α and PPAR γ and maintains pre-adipocytes in an undifferentiated state [87]. Research from the Marian lab has provided evidence that nuclear PG mediates adipogenesis by interfering with β -catenin/TCF transcriptional activity. Knockdown of desmoplakin in the cardiomyocyte cell line HL-1 led to translocation of PG to the nucleus and upregulation of adipogenic genes [89]. Moreover, Oil Red O staining detected lipid droplets confirming the presence of mature adipocytes in desmoplakin-deficient mouse hearts [89]. From these studies it was proposed that disruption of desmosome structure allows PG to leave the ICD and enter the nucleus, bind TCF/LEF thus suppressing β -catenin transcriptional activity and allowing activation of adipogenic genes.

A key question in the pathogenesis of ARVC is the cellular origin of the excess adipocytes. As adult cardiomyocytes are terminally differentiated and unlikely to de-differentiate to adipocytes, it has been proposed that a cardiac progenitor cell not fully committed to the myocyte lineage has the potential to differentiate into another lineage including adipocytes. Indeed, this may be the case as Marian's group performed genetic fate-mapping experiments using three cardiac lineage Cre transgenes to delete DP in different subsets of progenitor cells and compare the extent of adipocyte differentiation in the adult heart [90]. The authors discovered that adipocytes in the DSP mouse model of ARVC originate from second heart field cardiac progenitor cells. The same group went on to show co-expression of second heart field markers and adipogenic transcription factors in rare cells of human hearts from ARVC patients. Transgenic overexpression of PG in the heart is sufficient to induce fibro-fatty cardiac pathology in mice [90]. PG is found in the nucleus of the transgenic mice where it binds to TCF4 and suppresses Wnt/ β -catenin target genes. Conversely, expression levels of adipogenic factors Wnt5b and BMP7, which are normally inhibited by canonical Wnt signaling, increase in the PG transgenic hearts.

In a follow-up study the same investigators generated a transgenic model expressing the truncated form of PG seen in Naxos patients [60]. Similar to overexpression of full-length PG, the truncated PG resulted in cardiac pathology reminiscent of ARVC patients including adipogenesis. Cardiac progenitor cells (c-Kit $^{+}$) isolated from the transgenic hearts overexpressing either wild-type or truncated PG exhibit enhanced adipogenesis *in vitro* consistent with the investigators previous cell lineage tracing experiments described above. Moreover, c-Kit $^{+}$ progenitor cells isolated from PG-null embryos were resistant to

adipogenesis and expressed high levels of canonical Wnt target genes. Pharmacological inhibition of GSK3 β using 6-bromoindirubin-3'-oxime (BIO) reversed the pro-adipogenic transcriptional switch and prevented adipogenesis in the c-Kit⁺:Sca1⁺ cells consistent with activation of β -catenin transcriptional activity. Co-immunoprecipitation experiments demonstrated a decreased interaction between truncated PG and desmosomal proteins (DP and DSG2) consistent with the increased nuclear localization of PG [89]. The investigators conclude that the excess adipocytes seen in ARVC patients are dependent on PG inhibition of Wnt/ β -catenin signaling in cardiac progenitor cells. While transgenic studies have understandable limitations, this evidence provides a plausible molecular explanation for adipocyte differentiation in ARVC patients. Future genetic experiments such as knockin of the specific mutation into the endogenous PG gene will be required to demonstrate that physiological levels of mutant PG are sufficient to elicit the adipogenic phenotype.

The critical requirement for PG and β -catenin as linker proteins in the cadherin cell adhesion complex has complicated analysis of the cardiac-specific knockout mice as the combined protein level of these two closely related armadillo family members is carefully regulated by the cell. Ablation of either PG or β -catenin in the adult heart leads to upregulation of the other homolog, i.e. PG-deficient hearts have increased β -catenin [86] and vice versa [58]. Moreover, even reduction of PG as observed in PG hypomorph mice causes an increase in β -catenin levels [91]. The compensatory mechanism is posttranscriptional as no changes in β -catenin mRNA levels occur in these animal models [86, 91]. Interestingly, despite the increase in β -catenin in the PG mutant mice there does not appear to be increased β -catenin transcriptional activity at least as determined by the β -catenin/TCF reporters, TOP-gal or Bat-gal [88, 91]. The inability of PG to support hypertrophic growth in the stressed myocardium of β -catenin CKO mice is consistent with its weaker transcriptional activity. Alternatively, all available PG may be sequestered at the ICD in the absence of β -catenin thus it would not be available to translocate to the nucleus and activate gene transcription. Although not fully understood, the functional interdependence between β -catenin and PG is integral to preserving the structural integrity of the heart but fails to complement deficits in signal transduction. The lack of immunoblot data from human hearts makes it difficult to know if β -catenin is upregulated in ARVC patients carrying PG mutations. If upregulation of β -catenin is specific to murine models it may explain the lack of adipogenesis in the PG mutant mice.

PG and gap junction remodeling

The loss of large gap junction plaques from the termini of cardiomyocytes referred to as gap junction remodeling is commonly observed in many forms of heart disease including hypertrophy, ischemia, and dilated cardiomyopathies [92]. Gap junctions allow for electrical coupling of cardiomyocytes, ensuring the coordinated muscle contraction required for proper heart function. Molecular examination of human ARVC hearts revealed gap junction remodeling, likely contributing to the arrhythmogenic substrate [93]. Consistent with the human data, PG CKO mice exhibit decrease in Cx43-containing gap junction plaques at the ICD [86]. However, depletion of PG in the adult heart did not cause an apparent conduction abnormality [86]. Interestingly, β -catenin association with Cx43 was strengthened in PG deficient hearts [94], suggesting a protective function of β -catenin in PG CKO hearts. In this regard, β -catenin has been shown to be essential for microtubule-mediated connexon trafficking to the plasma membrane in HeLa cells [95]. Specifically, EB1, a microtubule plus end binding protein, has been found to facilitate delivery of Cx43 to the ICD by tethering microtubule plus ends to β -catenin. Depletion of β -catenin has a modest effect on gap junction remodeling compared to loss of PG [94]. This may be explained by the structural remodeling of the desmosome and subsequent cardiac remodeling observed in the PG CKO hearts that likely contributes to the more pronounced gap junction remodeling.

Removal of both PG and β -catenin (i.e. double knockout (DKO)) specifically from the heart causes severe conduction abnormalities and spontaneous lethal arrhythmias in these double mutant mice [94]. Moreover, a direct comparison of Cx43 expression in isolated cardiomyocytes from PG CKO and PG/ β -catenin DKO hearts reveals a further decrease of Cx43-containing gap junction plaques, consistent with the SCD phenotype in the DKO mice. The disassembly of the ICD in the PG/ β -catenin DKO hearts and the SCD are reminiscent of the cardiac-specific N-cadherin knockout model [96, 97]. Taken together, these studies demonstrate that deletion of a single cadherin linker protein, PG or β -catenin, in the mature adult heart is not sufficient to produce arrhythmias as the remaining arm protein compensates for loss of the other and stabilizes the remaining gap junctions [58, 86].

Conclusions

The importance of PG as a cell adhesion molecule critical for maintaining the structural integrity of the skin and heart is well-established from human genetic disease and murine models. In addition to its role in desmosome assembly, PG has been implicated in the modulation of the Wnt signaling pathway. Interest in the signaling capabilities of PG is increasing as a number of groups in different tissue systems attempt to elucidate the nuclear function of PG. Transgenic models attempting to recapitulate ARVC have shown that PG can translocate to the nucleus and inhibit canonical Wnt/ β -catenin signaling by sequestering TCF/LEF thus stimulating adipogenesis. On the other hand, there is compelling new data supporting PG as a direct regulator of Wnt target genes during keratinocyte differentiation. These results indicate the potential importance of PG as a modifier of canonical Wnt signaling either through suppression of β -catenin-mediated transcriptional activity or by direct positive or negative regulation of Wnt target genes. The mechanism(s) by which PG regulates transcriptional activity either directly or indirectly by sequestering TCF/LEF from binding to β -catenin may differ between keratinocytes and cardiomyocytes. Future studies with high resolution ChIP-chip molecular analysis are required to determine if PG can associate with target gene promoters in cardiomyocytes. Additionally, understanding how the cell senses changes in PG and β -catenin levels and the subsequent posttranscriptional compensatory mechanism will be of interest. As we learn more about the relationship between PG and β -catenin in the heart it may provide insight into novel therapeutic strategies for ARVC.

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Highlights

- Functional overlap and distinct roles of plakoglobin and β -catenin in the heart.
- Physiological consequences of human plakoglobin mutations.
- Role for nuclear plakoglobin in disease pathogenesis.

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Figure 1. Plakoglobin and β-catenin have similar structure

Schematic representation of plakoglobin and β-catenin protein including the 13 *armadillo* repeats. PG and β-catenin share many of the same binding partners as shown above (boxes). Percent amino acid similarity between the central 'armadillo' domain and N- and C-terminus are shown below.

Figure 2. Structural and signaling functions of β -catenin and plakoglobin

(A) β -catenin (β -cat) is found in adherens junctions where it functions as a linker protein between N-cadherin, α -catenins, and the actin cytoskeleton. (B) In contrast, PG participates in both adherens junctions and desmosomes. PG binds to desmosomal cadherins, desmocollin2 (DSC2) and desmoglein2 (DSG2) and mediates their interaction, via desmoplakin (DP) and plakophilin (PKP2), with intermediate filaments (IF). In addition to their structural roles as part of the mechanical junctions, both β -cat and PG are regulated by Wnt signaling. Wnt ligands bind to the Frizzled (Frz) receptor and signal the inhibition of glycogen synthase kinase 3 β (GSK3 β) through dishevelled (Dsh). If Wnt signaling is inactive, cytosolic β -cat and PG are degraded by the “destruction complex” composed of adenomatous polyposis coli (APC), axin and GSK3 β . Phosphorylation of β -cat and PG by GSK3 β target it for ubiquitination and subsequent degradation by the 26S proteasome. Following a Wnt signal, β -catenin and PG accumulate in the nucleus and bind members of the T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF) family of transcription factors to activate target gene expression. (B) PG has been implicated as both a positive and negative regulator of gene expression. In the latter case, PG may interfere with gene expression by competing with β -catenin for binding to TCF/LEF thus suppressing β -catenin/TCF/LEF target gene transcription.

Figure 3. Identification of *JUP* mutations in human patients

The known *JUP* mutations (protein designation) and their associated phenotypes are shown. The one dominant heterozygous mutation is shown above PG whereas the recessive homozygous mutations are shown below the protein.

Figure 4. Model of the molecular pathogenesis of ARVC

Cascade of events leading from desmosomal gene mutation to disease pathogenesis (adapted from [99]). The mutant desmosomal protein weakens the mechanical junction causing a cell adhesion defect which has multiple consequences on heart physiology. Electron micrographs show normal (PG WT) and abnormal (PG CKO) desmosome structure in the heart [86]. The loss of membrane integrity leads to myocyte cell death. The lost myocytes are then replaced by fibro-fatty tissue, a unique pathology observed in ARVC. It has been proposed that destabilization of the desmosome allows release of PG from the sarcolemma and its translocation to the nucleus. The adipocytes are thought to originate from a cardiac progenitor cell that undergoes a myogenic to adipogenic gene expression switch due to the ability of PG to sequester TCF/LEF thus inhibiting pro-myogenic Wnt/ β -cat signaling. Finally, altered mechanical junctions lead to diminished gap junction plaques at the ICD, a process called gap junction remodeling which is known to be pro-arrhythmic. Ventricular arrhythmia in an α MHC/Cre; PG^{flox/flox} (Mut) mouse is shown [88].

Table I

Genetic manipulation of armadillo proteins in the adult myocardium

Mouse model	Stress	Cardiac Phenotype	Reference
β -catenin LOF	None	Normal cardiac function, increase PG	[58]
β -catenin LOF	TAC	Block hypertrophy, decrease Wnt genes	[61]
β -catenin LOF	AngII	No change in hypertrophy response	[98]
β -catenin LOF	MI	Improve LV function, increase cpc	[63]
β -catenin HET	TAC	Block hypertrophy, increase fetal genes	[62]
β -catenin ^{Δex3} GOF	None	Cardiomyopathy, no change Wnt genes	[59]
β -catenin ^{Δex3} GOF	MI	No improvement in LV function, decrease cpc differentiation	[63]
β -catenin ^{Δex3} GOF	AngII	Block hypertrophy, decrease cardiac function	[98]
PG LOF	None	ARVC-like pathology except adipocytes	[86, 88]
PG Hypomorph	None	Normal cardiac function, increase β -catenin	[91]
PG GOF	None	ARVC-like pathology including adipocytes	[60]
PG ^{Naxos} GOF	None	ARVC-like pathology including adipocytes	[60]

LOF, loss-of-function; GOF, gain-of-function; HET, heterozygous; TAC, transverse aortic constriction; AngII, angiotensinII; MI, myocardial infarction; cpc, cardiac progenitor cells.