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Serine-threonine protein phosphatase regulation of Cx43 dephosphorylation in arrhythmogenic disorders

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Abstract

Regulation of cell-to-cell communication in the heart by the gap junction protein Connexin43 (Cx43) involves modulation of Cx43 phosphorylation state by protein kinases, and dephosphorylation by protein phosphatases. Dephosphorylation of Cx43 has been associated with impaired intercellular coupling and enhanced arrhythmogenesis in various pathologic states. While there has been extensive study of the protein kinases acting on Cx43, there has been limited studies of the protein phosphatases that may underlie Cx43 dephosphorylation. The focus of this review is to introduce serine-threonine protein phosphatase regulation of Cx43 phosphorylation state and cell-to-cell communication, and its impact on arrhythmogenesis in the setting of chronic heart failure and myocardial ischemia, as well as on atrial fibrillation. We also discuss the therapeutic potential of modulating protein phosphatases to treat arrhythmias in these clinical settings.

Keywords

Protein phosphatase; Connexin 43; Dephosphorylation; PP1; PP2A; Calcineurin

1. Introduction

Cell-to-cell electrical coupling (intercellular coupling) is an essential form of electrophysiological communication between adjacent myocytes occurring mainly via gap junction channels in the heart. Conduction slowing from decreased gap junctional coupling (as well as from decreased depolarizing currents) can lead to reentry that underlies lethal ventricular arrhythmias in the setting of heart failure (HF), myocardial ischemia and atrial fibrillation (AF) [1–6].

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Credit author statement

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Gap junctional channels, composed of connexins, are specialized membrane structures between adjacent myocardial cells [7,8]. Connexins assemble into hexameric pores known as connexons, which integrate into the cell membrane forming gap junction channels to facilitate exchange of molecules between adjacent cells to allow for intercellular electrical and chemical communication [9–13]. Connexins have four transmembrane domains with two extracellular loops (EL), one cytosolic loop, and a N-terminus and C-terminus. Connexins also interact with scaffolding proteins at the C-terminus, and may play a role in signaling pathways and cell regulation [14–17]. Thousands of gap junction channels assemble to form macromolecular complexes known as gap junction plaques, located at the intercalated discs between adjacent myocytes, that facilitate exchange of molecules between cells as part of gap junctional intercellular communication.

Gap junctional channels open or close in response to numerous triggers, including changes in transmembrane potential, changes in intracellular or extracellular ion concentrations, or alterations in phosphorylation status of connexin proteins [14–18]. Connexins are phosphoproteins and their post-translational phosphorylation influences the functional status of intercellular coupling, and dysregulation of the connexin phosphorylation occurs under various pathological conditions. An extensive number of protein kinases regulates connexin phosphorylation, primarily at serine and threonine sites, while only a few protein phosphatases are involved in modulating the phosphorylation state of connexins via protein dephosphorylation [19]. Much is known about these kinases, their key phosphorylation sites, and their potential as therapeutic targets [20]. However, understanding of the role of protein phosphatases in the function of connexin remains an ongoing effort. The phosphatases function as holoenzymes with multiple regulatory units controlling the catalytic units [21], while the activity of phosphatases can be modified via post-translational modification [22].

Protein phosphatases directly catalyze the hydrolysis of the phosphorylated amino acid residue in a protein molecule substrate. Based on the substrate specificity, protein phosphatases can be divided into protein serine-threonine phosphatases, protein tyrosine phosphatases superfamilies and dual-specificity phosphatases. Because over one third of all cardiac proteins go through the reversible phosphorylation/dephosphorylation process [23], phosphatases affect a vast spectrum of physiological activities in the heart, including cell-to-cell communication, excitation-contraction [24], Ca handling [25,26] and metabolism [27]. The serine-threonine phosphatases include three major families – the phosphoprotein phosphatases family (PPP), the Mg^{2+} or Mn^{2+}-dependent protein phosphatases family (PMP), and the recently discovered aspartate-based phosphatases [22,28–30]. Protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A), and protein phosphatase 2B (PP2B, also known as calcineurin) are the well-studied PPP members, and are responsible for 90% of dephosphorylation activities in the human heart [31,32]. This review focuses on the current understanding of protein phosphatase (PPase) regulation of the phosphorylation state of Cx43, the most abundantly expressed connexin in both ventricle and atria, and on intercellular coupling underlying ventricular arrhythmias in the setting of HF and ischemia as well as on AF. We will also address the therapeutic potential of modulating protein phosphatases at the level of Cx43 to treat arrhythmias in these clinical settings.

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2. Connexin expression and phosphorylation in the heart

There are three major connexins in heart: Connexin 43 (Cx43), Connexin 40 (Cx40), and Connexin 45 (Cx45). Cx43 is the predominant ventricular gap junction protein, but is also expressed in atrial and endothelial cells. Connexin 40 (Cx40) and connexin 45 (Cx45) are found primarily in atria and the conduction system [18,33–35] and are less abundant overall in the heart. The conduction properties of cells are influenced by the relative amounts, composition, and distribution of these connexins [36–38]. The abundance of Cx43 proteins is critically regulated at the transcriptional level. The Cx43 promoter harbors a number of binding sites of transcription factors including SP1, AP-1, CREB, c-Myc, HSP90, retinoic acid, and STAT [39–41]. Several transcriptional factors have been reported to enhance the Cx43 promoter activity and thus increased Cx43 expression in non-myocytes. For instance, several kinases including PKC, P38, ERK1/2, and JAK can activate the Cx43 promoter via the recruitment of transcriptional factors such as AP-1, SP1, STAT to upregulate the expression of Cx43 [42–45]. Interestingly, phosphoinostide-3 kinase (PI3K)/Akt signaling also activates the Cx43 promoter, but it does so by enhancing the binding of β-catenin to the Cx43 promoter [46,47]. Overall, a number of signaling pathways have been found to be involved in Cx43 expression via transcriptional regulation in non-myocytes. It is notable that Cx43 down-regulation in cardiac myocytes is a common feature in diseased and aged hearts [19,48–52]. However, current understanding regarding the underlying mechanisms of reduced Cx43 gene expression in myocytes remain limited. The Ai Lab recently reported for the first time that c-jun N-terminal kinase, an important member of MAP kinase, directly suppresses the promoter activity of Cx43 in myocytes via increased binding of c-jun to the promoter region, and consequently downregulates the mRNA and protein expression of Cx43 under stressed conditions such as advanced age in both humans and animal models [49]. Notably, Cx43 dephosphorylation has been found to be frequently linked to reduced Cx43 expression under certain pathological conditions. The involvement of phosphatases in transcriptional regulation of Cx43, however, is completely unknown and thus further investigations are needed.

Connexins, including Cx43, are known to be regulated by a number of post-translational modifications including phosphorylation, ubiquitination, sumoylation, S-nitrosylation, palmitoylation, hydroxylation, acetylation, methylation, and γ-carboxyglutamination [53], which ultimately modulate the function of gap junction channels. Extensive studies show that post-translational phosphorylation of Cx43 critically influences intercellular coupling through gap junction remodeling under pathological conditions. The function of Cx43, including channel conductance, can change in response to phosphorylation of at least 17 serine and 2 tyrosine sites located at the C-terminus. These include the serine sites S297, S306, S325, S328, S330, S365, S368, and the more recently characterized S282 [54,55]. Cx43 phosphorylation occurs via several kinases, including protein kinase A (PKA), protein kinase C (PKC), casein kinase 1 (CK1), mitogen-activated protein kinase (MAPK), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and Src kinases [14–16,20,23,32,33,48,56–59], and many of the kinases colocalize with Cx43 as part of the Cx43 macromolecular complex (interactome or connexome) [53,60–62]. Studies showed that PKA activation can increase or decrease conductance and alter cell-to-cell communication, while

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activation of PKC decreases gap junctional communication [63,64]. While MAPK promotes gap junction channel closure and destabilizes cytoskeletal anchoring, phosphorylation by CK1 promotes assembly and channel opening [49]. For instance, reduced phosphorylation of Cx43 either at Ser365 (PKA-dependent site) or Ser325/328/330 (CK1δ-dependent site) resulted in slowed cardiac conduction and enhanced arrhythmogenicity [65–67]. Transgenic knock-in mice with phosphomimetic Ser325/328/330Glu mutant Cx43 (S3E) was found to be resistant to ventricular arrhythmia with normal Cx43 channel function and normal calcium homeostasis in response to insults, while mutant Cx43 mice (S3A) with non-phosphorylatable Ser325/328/330 showed an enhanced arrhythmia risk [14,67]. Also, Cx43 dephosphorylation at S282 (PKA phosphorylation site) was found to mitigate ischemia/reperfusion-induced cardiac injury, and S282A mutation knock-in mice with non-phosphorylatable Cx43-S282 was reported to promote myocyte apoptosis and arrhythmias [54]. Moreover, recent studies indicate that Cx43 remodeling could mediate contractile dysfunction and arrhythmias via the opening of Cx43 hemichannels [68–70]. While numerous kinases have been shown to phosphorylate Cx43, only Akt, MAPK and PKC have been shown to regulate the activity of Cx43 hemichannels, and thereby potentially affect cardiac excitability. For instance, Akt (PKB) phosphorylation on Ser373 increases hemichannel function by enhancing the levels of surface Cx43 [71,72], while PKC's inhibit hemichannel activity by phosphorylation of Ser368 [73–77]. MAPK's have also been shown to promote hemichannel opening [78–80], but other studies show inhibition of hemichannel opening [81,82]. It is clear that further studies are needed to advance our understanding of the functional impact of MAPKs on gap junction channels.[83,84]

3. Phosphatases and Cx43 channel function

Although kinases are critical in regulating Cx43 phosphorylation, protein phosphatases are also important in maintaining the phosphorylation status by dephosphorylation of Cx43 proteins. In contrast to the growing literature on the role of phosphorylation on hemichannel function, little is known about the role of phosphatases on gap junction regulation [85].

Cx43 dephosphorylation, often in the setting of reduced Cx43 expression and redistribution, is a major contributor to altered intercellular coupling and slowing of conduction under certain pathological conditions [14–16,18,19,58,86–88]. On the other hand, dysregulation of the key protein phosphatases has been found in numerous pathologic settings including HF, myocardial ischemia, and AF. Cx43 can be dephosphorylated by a limited number of protein phosphatase, mainly the ubiquitous serine-threonine protein phosphatase – PP1, PP2A, and PP2B(calcineurin) [32,33,89]. While these phosphatases are present in the cytoplasm, PP1 [19] and PP2A [19,90] have been shown to colocalize with Cx43 [19,90], suggesting their local control of Cx43 phosphorylation state at the level of the gap junction. Inhibiting phosphatase activity was indeed found to improve intercellular coupling by improving the phosphorylation state of Cx43, supporting the critical role of PPase's in reduced intercellular coupling and arrhythmia development via Cx43 dephosphorylation [19,89,91].

It is known that the activity of PP1 can be specifically regulated by two endogenous PP1 inhibitors (heat-stable protein inhibitors), inhibitor-1 (I-1) and inhibitor-2 (I-2) [92–95], that are expressed in heart [96,97]. Likewise, PP2A has endogenous inhibitor proteins,

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inhibitor1PP2A (I1PP2A) and inhibitor2PP2A (I2PP2A) [98–100], that are also expressed in heart [97]. Intrinsic or extrinsic PPase inhibition, as well as modulation of upstream regulators of PPase activity, may be effective therapeutic approaches to reduce PPase activity, improve Cx43 channel function, and ultimately modify conduction. Unfortunately, there is nothing known about the effects of these endogenous PPase inhibitors on Cx43 and gap junctional conductance. Thus, future investigations are required. Calcineurin is another serine-threonine PPase, and its activity is Ca^{2+}-calmodulin-dependent. Studies suggest that calcineurin activation by raised intercellular [Ca^{2+}] reduces gap junctional conductance (G_j) via dephosphorylation of Cx43 at the Ser365 site, which is the result of calcineurin-mediated activation of PP1 [101]. Notably, phosphorylation of S365 is known to promote gap junction assembly and channel opening, while phosphrylation of S368 promotes channel closure. Since phosphorylation of S365 and S368 are mutually exclusive, enhancing the phosphorylation status of S365 could be a therapeutic intervention approach to improve the cell-to-cell communication under certain pathological conditions.

4. Phosphatases and Cx43 in heart failure

HF affects over 5 million people in the US, and nearly half of these patients die suddenly, primarily from ventricular tachycardia (VT) that degenerates into ventricular fibrillation (VF) [1]. In 3-dimensional cardiac mapping studies, the Pogwizd lab previously showed that spontaneously occurring VT in nonischemic HF (both in a rabbit HF model and in patients with nonischemic HF) is initiated and maintained by a nonreentrant mechanism such as triggered activity from delayed or early afterdepolarizations [2]. However, mapping in their rabbit HF model and in patients with nonischemic HF [102] revealed altered anisotropic conduction and conduction block that could underlie the development of reentry (especially during the transition from VT to VF) [3], and that was likely due to altered intercellular coupling.

Cx43 remodeling has been found to be critical in impaired intercellular coupling and enhanced reentrant arrhythmias in HF. Down-regulation of Cx43 expression occurs in HF models and in failing human hearts [7,19,38,51,52,86,103,104], with subepicardial Cx43 reduced more than midmyocardial and subendocardial Cx43 [52]. Moreover, Cx43 is redistributed from the intercalated discs (at end-to-end junctions) to the lateral sides (side-to-side junctions) in a process known as lateralization [105,106], and Cx45 levels are elevated [52,107–109]. In the arrhythmogenic rabbit model of HF and idiopathic dilated cardiomyopathy (IDCM, nonischemic HF) patients, Cx43 protein and mRNA levels were decreased in left ventricular (LV) myocardium [19]. In control rabbit LV, Cx43 was primarily in the phosphorylated state, but with HF there was a significant increase in dephosphorylated Cx43 (similar results were noted in human IDCM LV myocytes). Moreover, PPase's were found to dephosphorylate Cx43 [19], evidenced by increased amount of PP2A co-localized with Cx43 in HF, whereas colocalized PP1 was unchanged [19]. These changes in local expression were considerably different from changes in global expression, where total PP2A was decreased and total PP1 levels were increased in HF myocytes. This PP2A-modulated dephosphorylation of Cx43 led to decreased intercellular coupling, while was improved with the treatment of the PPase inhibitor okadaic acid (at a

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concentration that inhibited PP2A but not PP1) [110,111], suggesting decreased intercellular coupling in HF myocytes can be enhanced by PPase inhibition.

PAKs are another family of serine-threonine kinases (PAK 1 to 6) that phosphorylate a variety of substrates [112–114]. PAK1, PAK2, and PAK3 have been shown to play important roles in cardiac function [112–115]. PAK1, in particular, has been shown to co-localize with PP2A and to modulate PP2A activity [103,116–118]. PAK1 and activated PAK1 (pPAK1 that is phosphorylated at the Thr423 site) protein levels were enhanced with HF. We investigated the regulation of PAK1 in dephosphorylation of Cx43 via PAK1-regulated PP2A activity in HF [103]. While PAK1 colocalized with Cx43, and the levels of colocalized PAK1 and PP2A increased in rabbit and human HF, PAK1 overexpression in nonfailing myocytes increased PP2A activity, dephosphorylated Cx43, and decreased intercellular coupling, effects that were blocked by PP2A-specific inhibition with okadaic acid [103]. These findings suggest that enhanced PAK1 contributes to increased PP2A activation at the local level of Cx43 proteins in HF, resulting in increased Cx43 dephosphorylation that is associated with decreased intercellular coupling [103]. PAK1-mediated regulation of Cx43 through PP2A may therefore be used as a potential therapeutic approach for preventing ventricular arrhythmias in HF through improved intercellular coupling.

In HF, a number of studies have shown enhanced PP1 activity in the heart [119–123], but other studies demonstrated conflicting results [23,124–127]. Notably, most of these studies measured only the PP1 catalytic subunit (PP1c) and assessed global levels or activity, and did not explore its diverse set of interactors, which confer localization and substrate specificity to PP1. Chiang et al. [128] recently assessed the PP1 interactome during HF progression in mice. Affinity purification with anti-PP1c antibodies was followed by high-resolution mass spectrometry. Seventy-one PP1c interactors were quantified, and they demonstrated 9 PP1c interactors (Ppp1r7, Ppp1r18, and 7 novel interactors) with changes in their binding to PP1c strongly associated with HF progression. The interactors involved with PP1 activation at the level of Cx43 remain to be determined, but this novel approach could identify key local targets in the failing heart. To explore why there are decreased PP2A levels in HF, expression level of the PP2A catalytic subunits B56α and B56δ were assessed and found to be decreased in the failing heart [129,130]. B56α is a potential target to indirectly inhibit PP2A via its interaction with ankyrin-B [131,132]. Interestingly, mice deficient in B56α exhibited increased PP2A activity and slow conduction [133]. The role of B56α-PP2A and its association with ankyrin-B at the level of Cx43 remains unknown, but ankyrin-B is involved in the localization of B56α-PP2A, which could have effects on PP2A activity and, subsequently, Cx43 dephosphorylation. Future investigation is clearly needed in this regard.

5. Phosphatases and Cx43 in myocardial ischemia

Ventricular arrhythmias such as VT and VF occur within minutes of onset of myocardial ischemia [134,135]. Three-dimensional cardiac mapping during early ischemia showed intramural reentry in nearly 75% of cases of VT [134] as well as during the transition

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from VT to VF [135], from slow conduction likely arising from altered cellular coupling (as well as altered ion channel function) [136].

Downregulation of Cx43 expression occurs in myocardial ischemia in both humans and animal models [134,135,137,138]. However, the role of Cx43 dephosphorylation in intercellular uncoupling and arrhythmogenesis began with the seminal work by Beardslee et al. [138]. In the perfused rat heart, they showed progressive Cx43 dephosphorylation, with dephosphorylated Cx43 accumulation at sites of gap junctions, with a time course similar to ischemia-induced electrical uncoupling. Total Cx43 expression was unchanged over that time period, suggesting that accumulation of dephosphorylated Cx43 could underlie gap junctional uncoupling in that setting. With ischemia, Cx43 is found to redistributed from end-to-end to side-to-side (i.e. lateralization) and is mainly in the dephosphorylated state [50]. Considerable work has focused on specific Cx43 serine sites and their phosphorylation state during ischemia [139–141]. Axelsen et al. [142], for example, used a mass spectroscopy approach and identified 13 serine phosphorylation sites (3 of which were not previously described), and they found dephosphorylation at Ser306, Ser297, and Ser368, as well as phosphorylation at Ser330 in the ischemic heart [142]. More recently, Ser282 has been shown to have an important role in ischemia [54,55]. The role of protein phosphatases in ischemia-induced Cx43 dephosphorylation was reported in both isolated myocytes and isolated perfused heart. PP1 + PP2A inhibition with okadaic acid and calyculin (but not the PP2A inhibitor fostreicin) decreased dephosphorylated Cx43 accumulation at the intercalated discs [143]. PP1 inhibitors also improved gap junctional uncoupling during ATP depletion [91]. These findings point to an important role for PP1, but the fact that okadaic acid and calyculin did not completely prevent the ischemia-induced Cx43 dephosphorylation suggest that other phosphatases could also be involved. In support of this are the findings that calcineurin activity is increased in ischemic rat heart [144,145], and inhibition of calcineurin by cyclosporine A in rats prevented Cx43 dephosphorylation after myocardial ischemia; but the functional contribution of calcineurin in intercellular coupling remains to be determined [137]. Moreover, the role of PPase's was explored in studies of ischemic preconditioning (IP), where repeated short bursts of non-lethal ischemia and reperfusion are given prior to prolonged ischemic/reperfusion period. IP has been shown to prevent ischemia-induced Cx43 dephosphorylation [146–148]. The role of PP1, PP2A and calcineurin in preconditioned myocardium was studied in mini pigs subjected to IP + 90 min of low-flow ischemia. Totzek et al [90] found that Cx43 co-immunoprecipitated with PP2A, but not with PP1, and the amount of co-immunoprecipitated PP2A with Cx43 did not change with IP. These findings suggest that there may be species differences in the direct association of PPase's at the level of Cx43. While coprecipitated PP2A (with Cx43) did not change with IP+ ischemia, changes in PP2A activity at the level of Cx43 and/or contributions from other PPase's such as calcineurin could not be ruled out.

6. Phosphatases and Cx43 in atrial fibrillation

AF is the most common arrhythmia in clinical practice and is associated with a high risk of morbidity (stroke and HF) [149,150]. While aging is inevitable and the most prevalent risk factor for AF, various conditions (e.g. alcohol abuse, obesity, diabetes, HF, valvular diseases, myocardial ischemia) have been well recognized as independent AF risk factors [151–

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158]. The electrophysiological mechanisms underlying AF [159,160] include reentry (from slow conduction due to altered gap junctions, interstitial fibrosis, and sodium channels) [48,49,161–164] as well as non-reentrant activation from triggered activity due to delayed and/or early afterdepolarization as a result of altered Ca^{2+} handling [5,163,165–170]. Over the years, significant progresses have been made regarding the underlying mechanisms of altered gap junctions in AF and potential approaches for therapeutic interventions.

Atrial gap junctions, composed of Cx43 and Cx40, form specialized membrane channel structures that critically influence electrical and chemical signal propagation between adjacent atrial myocytes [171,172]. Atrial gap junction remodeling leads, at least in part, to slowed action potential propagation that facilitates arrhythmic reentry circuits [19,48,86,172,173]. However, gap junctional remodeling in atrial tissue from AF patients has been variably reported as an increase or a decrease in one or more connexins (Cx43 and/or Cx40), and as increased heterogeneity in the distribution of the connexins. Each of these results is dependent on comorbid cardiovascular diseases, the type of AF (paroxysmal or chronic), or the AF animal model used [174,175]. The Ai lab found significantly decreased Cx43 in aged atria from humans, rabbits, and mice [48,49,176]. Moreover, reduced Cx43 was also reported in the tachy-pacing induced AF model of swine. Overexpression of Cx43 using atrial gene transfer corrected Cx43 downregulated-caused slow conduction and AF propensity [162], suggesting the critical role of Cx43 in AF.

While growing evidence has supported the functional contribution of Cx43 in reentrant arrhythmias and AF, the underlying mechanisms of altered Cx43 and its functional impact in stressed or diseased hearts remains unclear. The c-jun N-terminal kinase (JNK) is a stress-response protein kinase, one member of the mitogen-activated protein kinases (MAPKs) family, which is known to be critical in the development of cardiovascular diseases including HF, hypertrophy, and atherosclerosis [177,178]. The Ai lab recently elucidated the pivotal roles of the stress kinase JNK in suppressing Cx43 expression and enhancing abnormal triggered Ca^{2+} activities, both of which promote the formation of the arrhythmogenic substrate of AF [48,49,165–167]. Specifically, activated atrial JNK downregulated Cx43 expression via suppressed transcriptional activity by increased binding of the JNK-downstream transcriptional factor c-Jun to the Cx43 gene promoter, which consequently led to impaired intercellular coupling and slowing of conduction in the atrium [49]. Meanwhile, activated atrial JNK also drove a pathogenic kinase-to-kinase crosstalk between JNK kinase and the ‘pro-arrhythmic kinase’ CaMKIIδ in the control of intercellular Ca^{2+} signaling and consequently Ca^{2+}-mediated aberrant triggered activities. Overall, these studies provided direct evidence for the causative action of JNK in atrial arrhythmogenic remodeling and a novel molecular mechanism underlying JNK-regulated Cx43 gene expression and impaired intercellular coupling. It also supports that JNK kinase activity modulation could be a potential therapeutic approach for improved atrial conduction and AF prevention in the aged heart. To date, the direct regulation of JNK on the phosphorylation status of Cx43 remains unknown. However, it is interesting to note that the JNK activator anisomycin has been shown to stimulate JNK activity by inducing ubiquitination and degradation of the dual-specificity phosphatase 8 (DUSP8, M3/6) [179]; and other DUSP’s such as DUSP1 inhibit JNK in heart [180]. The relationship between JNK kinase and PPases in Cx43 is clearly worthy of further investigation.

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Cx43 dephosphorylation and lateralization, as well as reduced coupling were observed in atria from patients with AF and in dilated atria from infarcted rats [181]. However, little is known about the role of phosphatases at the level of Cx43 in AF. Altered expression and activity of protein phosphatases in human chronic AF have been reported, although reported findings are inconsistent [119,182,183]. One study reported increased PP2A but not PP1 [119,182], while another study found increased PP1 and PP2A activity along with hyperphosphorylated inhibitor 1 of PP1 (I-1) in atria from chronic AF patients [183]. In addtion, atrial samples from chronic coronary artery disease patients showed expression of I1PP2A and I2PP2A, and a significant decline in PP2Ac and I2PP2A in older patients [97]. In the end, it remains to be determined whether alteration in PP1 and PP2A activity and endogenous inhibitors I-1, I-2, I1PP2A and/or I2PP2A could modulate Cx43 phosphorylation and channel function at the level of Cx43 proteins, and contribute to development of AF.

7. Therapeutic implications of phosphatase modulation on Cx43 and future studies

PP1 and PP2A can be directly inhibited by small molecule serine/threonine phosphatase inhibitors. Although some of these inhibitors are selective for PP1 and/or PP2A, they all may also inhibit PP4, PP5, and PP6 to an extent, which may have undesirable effects [184]. The most selective and widely available PPase inhibitors are okadaic acid, calyculin A, and fostreicin. However, broad-acting phosphatase inhibitors such as okadaic acid and calyculin (that inhibit both PP1 and PP2A) are known to have significant toxicity precluding any therapeutic use. Fostreicin has been reported to be highly selective for PP2A over PP1 [185]. While fostreicin did not reduce ischemia-induced Cx43 dephosphorylation [143], it was protective during myocardial ischemia [186–188]. It is of particular interest since it is being investigated as a cancer treatment for its antitumor activity in vivo. It showed in vitro activity against leukemia, lung cancer, breast cancer and ovarian cancer [185,189,190] that is thought to be due to PP2A's assumed role in regulating cell apoptosis by activation of cytotoxic T-lymphocytes and natural killer cells involved in tumor surveillance. Fostreicin has been safely administered to patients in pharmacokinetic studies and clinical trials, supporting its possible use in clinical applications [191]. Likewise, the more selective PP2A inhibitor LB-100 [192–194] is in clinical trials in cancer patients, and further attests to the feasibility for focused phosphatase inhibition as a viable therapeutic approach.

With regard to potential phosphatase modulation of Cx43, the peptide rotigaptide (ZP123), a derivative of antiarrhythmic peptide, has been shown to improve gap junctional conductance in vitro [195,196], and to have antiarrhythmic activity in vivo [197,198]. The mechanisms of rotigaptide's effects have remained unclear, and its decreased phosphorylation of serines 297 and 368 [142] on Cx43 could be due to effects on an unknown phosphatase. Unfortunately, rotigaptide is no longer in development, but the intriguing antiarrhythmic effects with rotigaptide [198–206], as well as that of a derivative, danegaptide (ZP1609 or GAP-134) [199,202], suggest that Cx43 phosphorylation state remains a viable therapeutic target for antiarrhythmic therapy for HF, myocardial ischemia, and AF.

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Another potential approach to phosphatase modulation of Cx43 is microRNA's. Protein phosphatases are regulated by microRNA's. In particular mirR-1 and miR-133 have been shown to contribute to arrhythmogenesis through changes in RyR2 dephosphorylation by PP2A, leading to abnormal Ca^{2+} handling by the sarcoplasmic reticulum [130,207]. Cx43 is a direct target of miR-1 [208–210], and miR-1 has been shown to modulate conduction through its effects on Cx43 [211]. Whether miR-1's effect on Cx43 is mediated by phosphatase activation requires further investigation.

A number of early studies including studies in knockout mice have suggested that gap junctions may not be the only means by which cells can electrically couple [212]. Recent work suggests that non-GJ-mediated coupling between cells (i.e. ephaptic coupling) could underlie intercellular transfer of electrical activation by means of electric fields within a confined extracellular space such as the perinexus, a perijunctional domain around the GJ's [213,214]. The beta subunit of Nav1.5 sodium channels play an essential role [215,216], and ephaptic coupling is sensitive to extracellular concentrations of Na, K and Ca ions [217,218]. If intact GJ's are required for bringing perinexal membranes in close enough proximity to allow for ephaptic signaling, interventions that maintain Cx43 gap junctions at the intercalated discs may be effective in preserving ephaptic signaling that would otherwise be lost. While several studies have shown effects on ephatic coupling independent of changes in Cx43 phosphorylation state [217,219], little is known about the direct effects of kinases and phosphatases on this non-GJ coupling mechanism. Further investigations are needed.

Taken together, the phosphorylation state of Cx43 at various serine, threonine and tyrosine sites can significantly impact Cx43 expression and function in various pathological states such as HF, myocardial ischemia, and atrial fibrillation. While much work has focused on a large number of interacting kinases, the functional role of phosphatases is worthy of future studies. Moreover, the therapeutic potential of modulating phosphatase activity at the level of the gap junction should be further explored as a potential targeted antiarrhythmic strategy.

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