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Protein kinases in cardiovascular diseases

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Abstract

Cardiovascular disease (CVD) remains the leading cause of death worldwide. Therefore, exploring the mechanism of CVDs and critical regulatory factors is of great significance for promoting heart repair, reversing cardiac remodeling, and reducing adverse cardiovascular events. Recently, significant progress has been made in understanding the function of protein kinases and their interactions with other regulatory proteins in myocardial biology. Protein kinases are positioned as critical regulators at the intersection of multiple signals and coordinate nearly every aspect of myocardial responses, regulating contractility, metabolism, transcription, and cellular death. Equally, reconstructing the disrupted protein kinases regulatory network will help reverse pathological progress and stimulate cardiac repair. This review summarizes recent researches concerning the function of protein kinases in CVDs, discusses their promising clinical applications, and explores potential targets for future treatments.

Keywords: Protein kinases; Signal transduction; Cardiovascular diseases; Phosphorylation

Introduction

Cardiovascular diseases (CVDs) include several different pathologies, such as heart failure, ischemic heart disease, ischemia/reperfusion injury, arrhythmia, cardiomyopathies, and diseases of blood vessels such as hypertension and atherosclerosis. Despite advances in treatment and prevention, CVDs remain the leading cause of death worldwide and the most common cause of mortality in China, accounting for 40% of annual deaths.[1] Therefore, novel therapeutic strategies are still required. Fortunately, recent studies have suggested a variety of potential cardiac repair and function preservation treatments, including cell transplantation, gene reprogramming, and the regulation of functional signaling pathways. The role of protein kinases in signal pathways has also been confirmed.

Protein kinases belong to the kinase superfamily and are responsible for modulating cellular function through cascades of substrate phosphorylation and activation. Five hundred and eighteen human protein kinases have been identified since 1959, when the first protein kinase was purified.[2] According to the specific amino acid residue of their substrates, these kinases can be classified into three central subgroups:[3] serine/threonine kinases (STKs), tyrosine kinases (TKs), and dual-specificity kinases. In an activated state, human protein kinases share a similar catalytic structure. Since discovering the vital role of protein kinases in regulating cardiac metabolism, programmed cell death, transcription, and cell contractility, evidence has accumulated to show that protein kinases are significantly involved in the pathogenesis of CVDs. Fan *et al*,[4] for instance, showed that checkpoint kinase 1 (CHK1) significantly stimulates cardiomyocyte (CM) proliferation in neonatal mice hearts, and CM-targeting CHK1 overexpression in adult hearts was supposed to be a promising strategy for myocardial repair post-ischemia injury.

A significant amount of research has been conducted on pharmacological or gene therapies targeting protein kinases in the field of CVD due to the importance of protein kinases and protein phosphorylation in preventing CVD. To explore more potential therapeutic targets, we review the structure and function of protein kinases and analyze their role and mechanism in cardiovascular pathologies.

Structure and function of protein kinases

By phosphorylating substrates, protein kinases regulate a variety of cellular functions and biological activities. Previous studies have shown that a single protein kinase is encoded by several genes, whereas a single gene can also encode multiple protein kinase isozymes. Cloning strategies play an essential role in discovering and identifying

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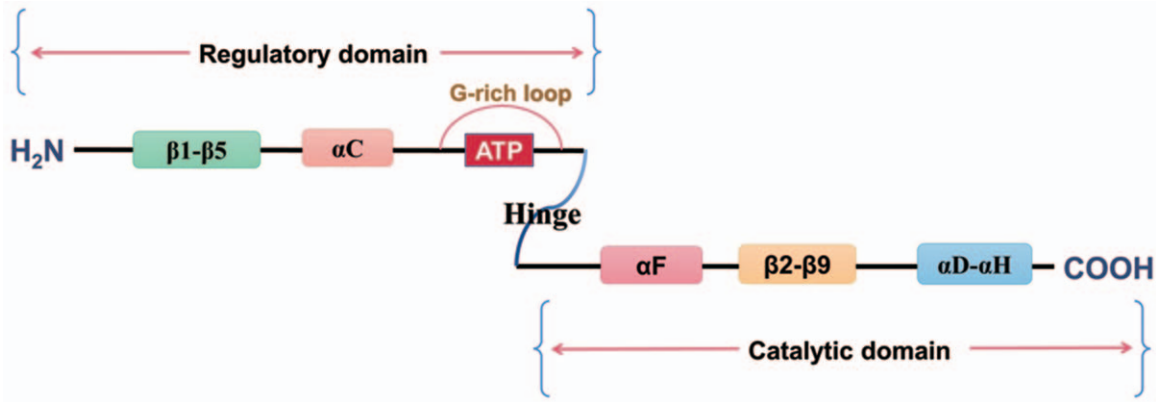

Figure 1: Schematic representation of the primary structure of protein kinases.

protein kinases because of the significant similarities between the catalytic domains of protein kinases. Though different protein kinases are closely related, they still distinguish themselves through numerous primary sequences and structural characters.

Structure of protein kinases

According to the nature of the phosphorylated-OH group of protein kinases, scientists identified protein kinases as protein-TKs (90 members), protein-STKs s (385 members), and tyrosine-kinase like proteins (43 members). As eukaryotic protein kinases, their catalytic domain consists of two mutual subdomains: the C-lobe and the N-lobe [Figure 1]. Between the two subdomains is the adenosine triphosphate (ATP) adenine ring beneath the G-rich loop, and they are connected by a peptide stand, which creates an active site consisting of two pockets that serve as catalytic residues.[5] The residue "gatekeeper" and conserved lysine residue control access to the back pocket. C-lobe plays a significant role in binding protein or peptide substrates and nucleotides.[5] The opening and closing of protein kinases are controlled by the catalytic and regulatory machinery attached to the C-lobe. Involved in most interactions, N-lobe mainly consists of a five stranded antiparallel β-sheet and a conserved αC-helix and is connected to the F-helix of C-lobe through the αC-β4 loop.[6] Besides their catalytic domains, kinases also possess non-catalytic domains that allow attachment of substrates and recruitment of other signaling molecules.

Although the activation segment of different activated protein kinases is similar and remarkably conserved, the inactive state is different. The interconversion of active and inactive conformations is determined by domain interaction alteration, usually triggered by signals. R-spine structure largely determines whether a protein kinase is activated or not. One example is the inactivation of AKT (protein kinase B). The Asp-Phe-Gly (DFG) phenylalanine position, which was one of the components of the R-spine, flips over and holds the position occupied by the ATP adenine ring in the C-spine in the active conformation.[7] As with many other kinases, inactive conversion did not involve the movement of DFG motifs, such as Src and cyclin-dependent kinase 2 (CDK2). The R-spine of Src and CDK2 is broken due to the displacement of the C-helix residue.[8]

Function and regulation of protein kinases

Through the phosphorylation of a series of substrates and interaction with different signaling pathways, protein kinases regulate cell survival and proliferation, programmed cell death, such as apoptosis, metabolism, and other important biological activities. For instance, several protein kinases, including calcium/calmodulin-dependent protein kinases (CaMK) and protein kinases A (PKA) phosphorylate phospholamban (PLN), which is the crucial regulator of sarcoplasmic reticulum (SR) pumping activity and will thus affect the myocardial contractility.[9] Besides, glycogen synthase kinase-3 (GSK-3) inhibits glycogen synthesis and thus reduces cardiomyocytes' energy supply through the phosphorylation of glycogen synthase.[10] Furthermore, by regulating the myocardin-related transcription factor, Rho-associated protein kinase (ROCK) promotes serum response factor binding, which leads to profibrotic genes activation and cardiac fibrosis.[11]

Activation of protein kinases is crucial to cellular activity, but it only occurs when corresponding signals or stimuli are present. The activation of most receptor protein-TKs depends on ligand binding, dimerization, and phosphor-ylation of the activation segment. The CDK family is activated by their cognate cyclins, whereas calcium-calmodulin complexes activate CaMK. Another class of kinases, such as cyclic nucleotide-regulated protein kinases, are activated by second messengers, but protein kinase C is activated by diacylglycerol. To summarize, the mechanisms by which protein kinases are activated are diverse and complex.

Protein kinases in cardiovascular diseases

A broad spectrum of CVDs involves the role of protein kinases. Several well-studied protein kinases will be discussed in this review, emphasizing their function in diseases. At the same time, some of the latest research developments will be discussed as well. The function and underlying mechanisms of protein kinases in CVDs were briefly summarized in Table 1.

Table 1: List of the protein kinases associated with cardiovascular diseases and their mechanism of action.

Disease model	Protein kinases	Model/ species	Functions	Mechanism	Reference
HF	CaMKII	Mouse	Contractile dysfunction↑	Phosphorylates PLN to activate SERCA2a and decrease intracellular Ca^{2+}	[12]
			HF progression↑	Inhibits HDAC4 nuclear exit to induce cardiac remodeling	[22]
			Hypertrophy↑	Degrades UBE2T to promote DNA damage accumulation	[24]
	AMPK	Rat	HF progression↓	Alleviates protein O-GlcNAcylation and maintain protein quality control	[27]
		Mouse	HF progression↓	Interacts with PINK1 at Ser495 to regulate mitophagy	[29]
	AKT	Mouse	Hypertrophy↑	Promotes protein metabolism through phosphorylation of GSK3β and mTOR, inhibits FOXO3 to strengthen cell growth and protein catabolism and inhibits CEBPβ to enhance CITED4 function of proliferation-promotion	[12,13,15,16]
	mTOR	Mouse	Hypertrophy↑ HF progression↑	Activates S6K1 and inhibits 4EBP1to promote protein synthesis and suppresses autophagy	[14,26]
	PKA	Mouse	Hypertrophy↑	Phosphorylates PLN to regulate Ca^{2+}-leak and regulates contractile proteins	[12,25]
	PI3K	Mouse	Hypertrophy↑	Actives AKT and MEK, thus promoting downstream metabolism and apoptosis-related pathways	[12,17]
	ERK	Mouse	Hypertrophy↓	Activates downstream anti-apoptotic proteins such as IGF-1 to regulate apoptosis	[18]
	GSK-3β	Mouse	Hypertrophy↑	Suppresses eIF2Be and reduce protein synthesis	[12]
	PKCα	Mouse	Hypertrophy↑	Phosphorylates PPP1R1A to alleviate PLN phosphorylation	[20]
	PKG	Mouse	Hypertrophy↓	Inhibits GPCR signal transduction to reduce cardiac hypertrophy	[28]
	SPEG	Rat	HF progression↓	Interacts with SERCA2a to regulate Ca^{2+} homeostasis	[21]
	PINK1	Mouse	HF progression↓	PINK1, phosphorylated by AMPK at Ser495, regulates mitophagy	[29]
	HIPK2	Mouse	HF progression↓	Interacts with ERK pathway to regulate cell apoptosis	[30]
Atherosclerosis	AKT	Mouse, human	AKT1↑atherosclerosis↓ AKT2↑atherosclerosis↑	Deficiency of Akt2 promotes M2 differentiation to inhibit plaque initiation and development	[41]
		Mouse, human	AKT1↑atherosclerosis↓	Suppresses Bad and Caspase and promotes genes including MDM2 and IKK to inhibit macrophage apoptosis	[46]
	P38	Human	Atherosclerosis↑	Regulates inflammatory activity, EC permeability and SMC apoptosis by regulating E-selection and VCAM-1 expression	[36-38]
	CaMKII	Mouse	Necrotic atherosclerotic plaques↑	Promotes ATF6 overexpression and activate MerTK to promote plaques development	[47]
	mTOR	Mouse	Atherosclerosis↓	Promotes histone acetylation and genes expression to support M2 phenotype, which is negatively associated with plaque development	[43]
	MerTK	Mouse	Necrotic atherosclerotic plaques↑	Activated by ATF6 and mediates necrotic atherosclerotic plaques development	[47]
	CK2	Rat	Atherosclerosis↑	Inhibits PRH expression to promote SMC accumulation	[35]
MI	mTOR	Mouse	CM proliferation↑	Mediated by integrin β3 to regulate autophagy-related genes and GSK-3βto promote CM proliferation and reduce CM death post injury	[59,60]
	ERBB2	Mouse	CM proliferation↑	Interacts with proliferation-related pathways including ERK, AKT, GSK-3β and activates YAP to regulate CM EMT-like response	[51,52]
	AKT	Mouse	CM proliferation↑	Phosphorylated GSK-3β at Ser9	[10]
	ERK	Rat	CM proliferation↑	Regulated by upstream proliferative factors including ECRAR and IL-13	[53]
	GSK-3β	Mouse	CM proliferation↓	Phosphorylated GSK-3β increases cyclin-D1 and inhibits β-catenin degradation	[10]
	ROCK	Mouse	Cardiac function↑ scar mass↓	Increases N-cadherin and integrin β1expression and improves the repair effects of hiPSC-CM transplantation	[63]
	P38	Rat	CM proliferation↓	Regulates mitosis-related genes such as cyclin A	[61]
	MerTK	Mouse	Cardiac function↑ scar mass↓	Interacts with STAT3 and ERK to promote osteopontin production of macrophages	[65]
	ILK	Mouse	Cardiac function↑ scar mass↓	ILK knockdown attenuates NF-κβ activation and enhances EPC exosomes' function	[64]
	CDK2	Rat	CM proliferation↑	Binding with cyclinB1 to promote CM cell cycle re-entry	[49]
	CDK9	Zebrafish	CM proliferation↑	Acting as proliferative gene binding partner to promote CM proliferation	[50]
	CHK1	Mouse	CM proliferation↑	Activates the mTORc1/P70S6K pathway to mediate CM proliferation	[4]
Hypertension and PAH	ERK	Human	Blood pressure↑	Increase intracellular ca^{2+} of SMCs to promote excessive contraction and promotes c-fos, AP-1 and HIF-1 expression to stimulate SMC proliferation	[69-72]
	ROCK	Rat, human	Blood pressure↑	Promotes SMC contraction, inhibits NO production and regulates sympathetic nervous system tone	[66-68]
	AKT	Human	Blood pressure↑	Promotes HIF-1 expression to stimulate SMC proliferation	[71,72]
	JNK	Mouse, human	Blood pressure↑	Activates mTOR and promotes c-jun and AP-1 expression to stimulate SMC proliferation	[13,70]
IRI	CaMKII	Rat	Reperfusion injury↑	Opens mPTP to promote myocardial necroptosis	[76]
		Mouse	Reperfusion injury↑	Activates NF-κβ signaling to trigger inflammation response	[79]
	GSK-3β	Mouse, rabbit	Reperfusion injury↓	Phosphorylates AMPKα and downstream autophagic proteins	[80]
	MAPK	Mouse	Reperfusion injury↓ cardiac function↑	Phosphorylates connexin 43 to regulate mPTP opening	[75]

(*continued*)

Table 1
(continued).

Disease model	Protein kinases	Model/ species	Functions	Mechanism	Reference
	ε-PKC	Rat	Reperfusion injury↓ cardiac function↑	Not known, possibly related with apoptosis	[82]
	DNA-PKcs	Mouse	Reperfusion injury↑	Degrades BI-1 to promote mitophagy	[78]
	CK1	Mouse	Overexpression mediates cardioprotection	Phosphorylates connexin 43 to regulate mPTP opening	[75]
	JAK	Pig	Reperfusion injury↓ cardiac function↑	Phosphorylates STAT3 at Tyr705 to mediate cardioprotective effect	[74]
	STAT3	Pig	Reperfusion injury↓ cardiac function↑	Preserves complex 1 respiration and improves calcium retention capacity	[74]
	SNRK	Mouse	Reperfusion injury↓ cardiac function↑	Regulates UCP3 to ameliorate mitochondrial efficiency	[77]
	GRK2	Rat	Reperfusion injury↓ cardiac function↑	Regulates fibrotic gene expression and neutrophils infiltration	[81]
HCM	AKT	Mouse, human	Cardiac hypertrophy↑ cardiac function↓	Phosphorylates FOXO3 to upregulate YAP expression	[83]
	ERK	Human	Cardiac hypertrophy↓ cardiac function↑	Regulates the RAF1 mutation-related myofibril disarray	[86]
	CK2α1	Mouse	Cardiac hypertrophy↑ cardiac function↓	Phosphorylates HDAC2 at S394A to regulate gene reprogramming in HCM	[84]
	MEK	Human	Cardiac hypertrophy↓ cardiac function↑	Regulates the RAF1 mutation-related myofibril disarray	[85]
DCM	ERK	Mouse	Cardiac dilatation with LMNA mutation↑	Phosphorylates FHOD1 and FHOD3 to negatively regulate nuclear movement	[93]
	JNK	Mouse	Cardiac dilatation with LMNA mutation↑	Regulates expression of genes encoding sarcomere structure and cardiomyofiber organization	[91,92]
	GSK-3β	Mouse	Cardiac dilatation↑	Regulates DNA synthesis and cell apoptosis	[90]
	AMPK	Mouse	Cardiac dilatation↑	Phosphorylates troponin I to regulate myocardial contractility and Ca^{2+} sensitivity	[89]
	ILK	Mouse	Cardiac function↑ mechanotransduction↑	Regulates SERCA2a/PLN phosphorylation to conduct contractility	[88]
Arrhythmia	CaMK	Mouse, human	AF incidence↑	Phosphorylates multiple membrane ion channels to regulate membrane excitability and mediates increased SR Ca2+-leak	[22,96]
	AMPK	Rat, dog	AF incidence↓	Regulate activity of membrane ion channels and atrial gap junction proteins to destabilize the reentry rotors	[98]
		Mouse, rat	AF incidence↓	Regulate RyR2 and SERCA2a-dependent Ca2+-leak by inhibiting CaMKK and metabolism dysfunction	[98,99]
		Mouse	Heart rate↑	Regulatory g subunit confers energy sensor function and increase heart rate by binding adenine nucleotides	[106]
	PKA	Mouse	AF incidence↑	Activates RyR2 and voltage-gated ion channels to effect intracellular current	[103]
	SPEG	Mouse	AF incidence↓	Suppresses RyR2 activity and activate SERCA2a to reduce diastolic Ca^{2+}	[104]
	ROCK	Chicken	Atrioventricular conduction↑	Participates in the developmental process of atrioventricular node	[105]

↑: overexpression of protein kinases promotes the physiological or pathological process mentioned; ↓: overexpression of protein kinases inhibits the physiological or pathological process mentioned; 4EBP1: EiF4E-binding protein 1; AF: Atrial fibrillation; AKT: Protein kinases B; AMPK: Adenosine monophosphate-activated protein kinase; AP-1: Activator protein-1; ATF: Activating transcription factor; ATG-7: Autophagy-related gene; BI-1: Bax inhibitor-1; CaMK: Calmodulin-dependent protein kinases; CaMKK: CaMK kinase; CDK: Cyclin-dependent kinase; CDK2: cyclin-dependent kinase 2; CEBPβ: CCAAT/enhancer binding protein-β; CHK1: Checkpoint kinase 1; CITED4: CBP/p300-interacting transactivator 4; CK1: casein kinase 1; CK2: casein kinase 2; CM: cardiomyocyte; DCM: Dilated cardiomyopathy; DNA-PKcs: DNA-dependent protein kinase catalytic subunit; EC: endothelial cell; ECRAR: endogenous cardiac regeneration-associated regulator; eIF: Eukaryotic translation initiation factor; eIF2Bε: Eukaryotic translation initiation factor; EMT: Epithelial-mesenchymal transition; EPC: endothelial progenitor cell; ERK: Extracellular signal-regulated kinase; FHOD: Formin homology domain-containing proteins; FOXO3: Forkhead box protein O3; GPCR: G-protein-coupled receptor; GRK: G protein-coupled receptor kinase; GSK: Glycogen synthase kinase-3; HCM: Hypertrophic cardiomyopathy; HDAC: Histone deacetylase; HF: Heart failure; HIF-1: Hypoxia-inducible factor; HIPK: Homeodomain-Interacting Protein Kinase; hiPSC: Human-induced pluripotent stem cells; IGF: Insuline-like growth factor; IKK: IkappaB kinase; ILK: Integrin-linked kinase; IRI: ischemia reperfusion injury; JAK: Janus kinase; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinases; MDM2: Murine double minute 2; MEK: MAPK/ERK kinase; MerTK: Mer tyrosine kinase; MI: Myocardial infarction; mPTP: Mitochondrial permeability transition pore; mTOR: Mechanistic target of rapamycin; NF-κB: Nuclear factor κB; O-GlcNAcylation: O-linked-N-acetylglucosaminylation; PAH: Pulmonary arterial hypertension; PI3K: Phosphoinositide 3-kinase; Pim1K: Pim1 kinase; PINK: PTEN-induced putative kinase 1; PINK: PTEN-induced putative kinase 1; PKA: Protein kinase A; PKG: Protein kinase G; PLN: Phospholamban; PPP1R1A: Protein phosphatase 1 regulatory (inhibitor) sub-unit 1A; PRH: Proline-rich homeodomain; ROCK: Rho-associated protein kinase; RYR2: Ryanodine receptor; S6K1: Ribosomal protein S6 kinaseβ1; SERCA2a: Sarcoplasmic/endoplasmic reticulum Ca2+-ATPase; SMC: Smooth muscle cell; SNRK: Snf1-related kinase; SPEG: Striated muscle preferentially expressed protein kinase; STAT3: Signal transducer and activator of transcription; UBE2T: Ubiquitin-conjugating enzyme E2T; VCAM: Vascular cell adhesion protein 1; VCAM-1: Vascular cell adhesion protein 1; YAP: Yes-associated protein.

Heart failure and cardiac hypertrophy

Heart failure is the ultimate state of various heart injuries and is characterized by CM hypertrophy, reduced number of CMs, and cardiac fibrosis. Distinguished by different characteristics and underlying signaling pathways, cardiac hypertrophy can be classified into two types: physiological hypertrophy and pathological hypertrophy [Figure 2].

Phosphoinositide 3-kinase (PI3K)-AKT axis is one of the most well-studied protein kinases signal pathways in physiological hypertrophy, stimulating physiological CM

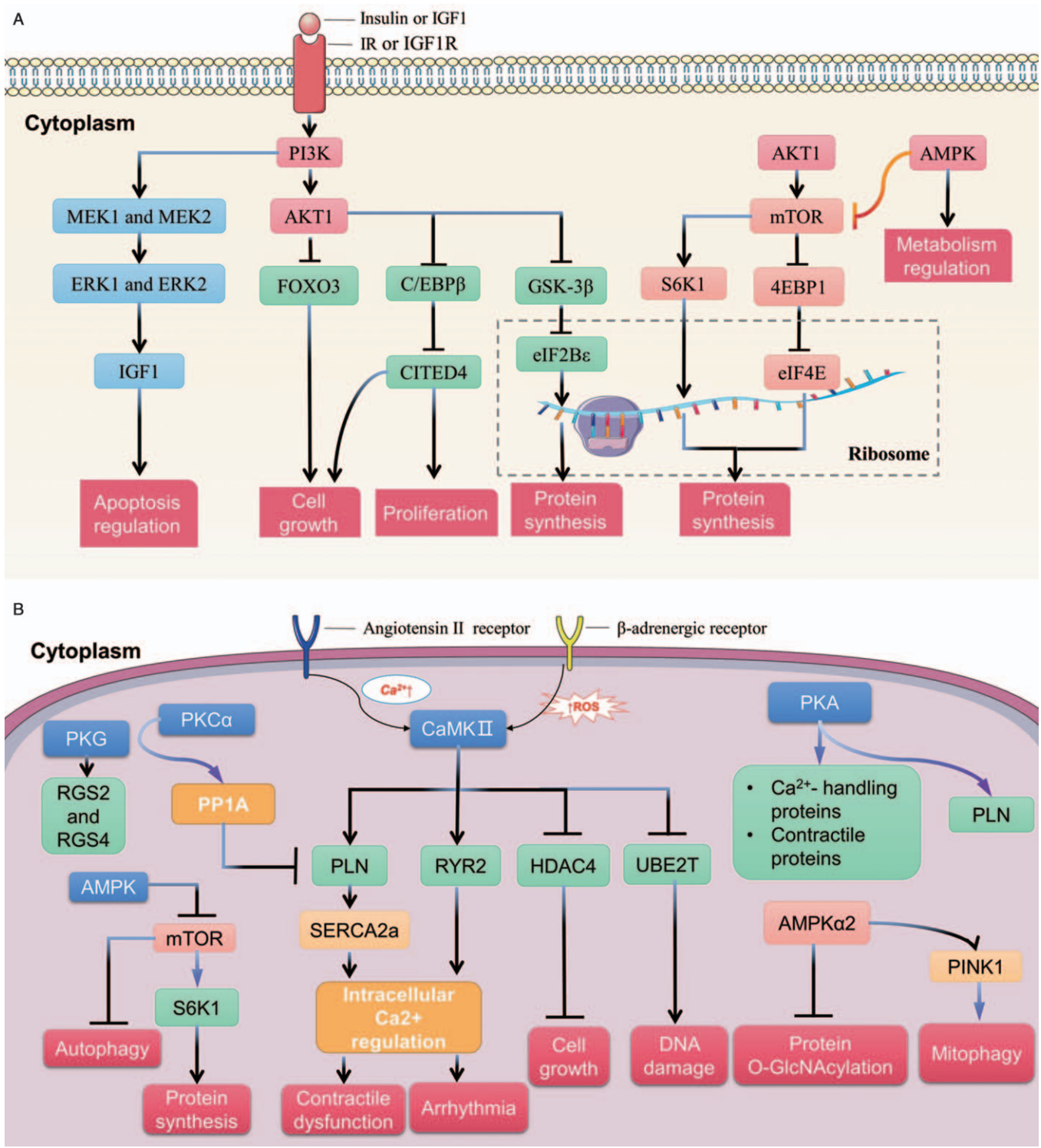

Figure 2: Protein kinases mediated signaling pathways in heart failure and hypertrophy. (A) The role of protein kinases in physiological hypertrophy. (B) The role of protein kinases in heart failure and pathological hypertrophy. 4EBP1: eiF4E-binding protein 1; AKT: Protein kinases B; AMPK: Adenosine monophosphate-activated protein kinase; C/EBPβ: CCAAT/enhancer binding protein-β; CaMK: Calcium/calmodulin-dependent protein kinases; CITED4: CBP/p300-interacting transactivator 4; eIF2Bε: eukaryotic translation initiation factor; eIF4E: eukaryotic translation initiation factor 4E; ERK: Extracellular signal-regulated kinase; FOXO3: Forkhead box protein O3; GSK: Glycogen synthase kinase; HDAC4: Histone deacetylase 4; IGF1: Insulin-like growth factor 1; MEK: MAPK/ERK kinase; mTOR: Mechanistic target of rapamycin; PI3K: Phosphoinositide 3-kinase; PINK: PTEN-induced putative kinase; PKA: Protein kinase A; PKC: Protein kinase C; PKG: Protein kinase G; PLN: Phospholamban; RGS: Regulator of G-protein signaling; RYR2: Ryanodine receptor; S6K1: Ribosomal protein S6 kinaseβ1; UBE2T: Ubiquitin-conjugating enzyme E2T.

growth through the regulation of protein metabolism, cellular proliferation, and apoptosis. First, activated by insulin receptor substrate 1 and IRS2, PI3K-AKT1 inhibits GSK3β[12] and activates the mechanistic target of rapamycin (mTOR)[13] to promote protein synthesis. Dephosphorylated GSK3β suppresses the eukaryotic translation initiation factor (eIF2Bε) expression whereas the activated mTOR stimulates ribosomal protein production by activating ribosomal protein S6 kinaseβ1 (S6K1) and inhibiting EiF4E-binding protein 1 (4EBP1).[14] Second, AKT1 suppresses the expression of transcription factor CCAAT/enhancer binding protein-β (C/EBPβ), thereby promoting hypertrophy by targeting the CBP/p300-interacting transactivator 4 to enhance cell growth and proliferation.[15] Further, AKT1 inhibits forkhead box protein O3 [16] expression to strengthen cell growth. In addition to conducting the growth-promotional signal through AKT, PI3K was also reported to mediate cardiac hypertrophy through regulating the mitogen-activated protein kinases (MAPKs) family.[17] Responding to physiological stimuli, PI3K activates MAPK/extracellular signal-regulated kinase (ERK) kinase (MEK)1/2 and the downstream ERK1/2 to promote CM hypertrophy by regulating downstream anti-apoptotic proteins such as insulin-like growth factor 1.[18]

With the progression of cardiac dysfunction, CM hypertrophy becomes maladaptive decompensation with multiple pathological processes, including cell death, Ca^{2+} handling dysregulation, and genes damage. The contractability of the heart is remarkably correlated with the pump activity of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), and cardiac PLN is a critical regulatory target.[19] Targeting PLN, protein kinase C (PKC)α phosphorylates protein phosphatase inhibitor 1, the activator of protein phosphatase 1 catalytic subunit alpha, thus alleviating the phosphorylation of PLN. PKA and CaMKII increase the PLN phosphorylation.[12,20] Dephosphorylated PLN attenuates SERCA2a activity, blocks Ca^{2+} from entering the SR and thus compromises the contractability of the heart. Alternatively, SERCA2a activity was also regulated by striated muscle preferentially expressed protein kinase (SPEG). By phosphorylating SERCA2a at Thr (484), SPEG enhanced the Ca^{2+}-transporting activity of SERCA2a, which means SPEG may be a novel therapeutic target for heart failure charactered with impaired calcium homeostasis.[21]

CaMKII is another crucial regulator in the progression of heart failure and pathological hypertrophy. In addition to phosphorylating PLN, activated CaMKII δ inhibits the nuclear exit of histone deacetylase 4,[22] induces cardiac remodeling and accelerates the transition from adaptive hypertrophy to heart failure.[23] Further, CaMKII δ9 phosphorylates and degrades ubiquitin-conjugating enzyme E2T (UBE2T), which disrupts UBE2T-dependent DNA repair and leads to the accumulation of DNA damage.[24] Similarly, another PLN-activator, PKA, directly targets Ca^{2+}-handling protein and contractile proteins, such as PLN and cardiac myosin binding protein C.[25] These effects cause arrhythmia and contractile dysfunction in response to sympathetic activation and increased catecholamine levels.[12]

Cardiac pathological hypertrophy is also accompanied by elevated protein synthesis, and the mTOR pathway is critically involved in these processes. However, the sustained activation of mTOR will lead to the suppression of autophagy and deterioration of the protein quality control mechanism.[26] Consistently, adenosine monophosphate-activated protein kinase (AMPK) is involved in the protein quality control by inhibiting protein O-linked-N-acetylglucosaminylation (O-GlcNAcylation).[27] There are still many other functional protein kinases, including protein kinase G,[28] PTEN-induced putative kinase 1,[29] and homeodomain-interacting protein kinase 2,[30] which are equally crucial in the progression of heart failure and CM hypertrophy, and their exact function and mechanisms are shown in Table 1 and Figure 2.

Several drugs targeting these functional protein kinases have been developed and tested. In the model of cardiac hypertrophy, mibefradil, rapamycin, and aliskiren are found to inhibit PI3K/Akt/mTOR-mediated autophagy to alleviate CM hypertrophy and reverse cardiac remodeling.[31-33] Similarly, metoprolol and bisoprolol inhibit PKC to reverse cardiac hypertrophy.[34]

Atherosclerosis

Atherosclerosis is progressive inflammatory progress and the primary cause of myocardial infarction (MI) and stroke. Several types of cells and kinases are involved in the progression of atherosclerosis, and we will use some of them to illustrate the complex regulatory network mediated by protein kinases.

Firstly, vascular smooth muscle cells (SMCs) are the most abundant cells in blood vessel walls. In atherosclerosis, vascular SMCs are crucial in thickening blood vessel walls through their growth, proliferation, and accumulation. Researchers reported in 2017 that both silencing and pharmacological inhibition of casein kinase 2 could significantly inhibit the cell cycle progression of vascular SMCs and prevent SMC accumulation through the activation of the proline-rich homeodomain.[35] Additionally, p38 was also identified to stimulate SMC apoptosis, which will lead to plaque destabilization and an increased risk of plaque rupture in advanced atherosclerosis.[36]

Alternatively, p38 is critically involved in the regulation of endothelial cells as well. The overexpression of p38 increased the expression of cell adhesion molecules E-selection and vascular cell adhesion protein 1,[37] strengthening the attachment of inflammatory cells to endothelial cells and the related inflammatory response. Furthermore, p38 also regulates endothelial cell permeability by increasing interleukin-6 expression.[38] The described functions of p38 in endothelial cells are strongly associated with a weakened protective barrier and regulatory function for underlying tissues. Recently, the use of hydroxytyrosol and epicatechin gallate was reported to suppress inflammatory processes and prevent atherosclerosis by inhibiting p38 phosphorylation.[39,40]

Last, the most critical cells in early atherosclerotic lesions and unstable plaques are macrophage cells and derived

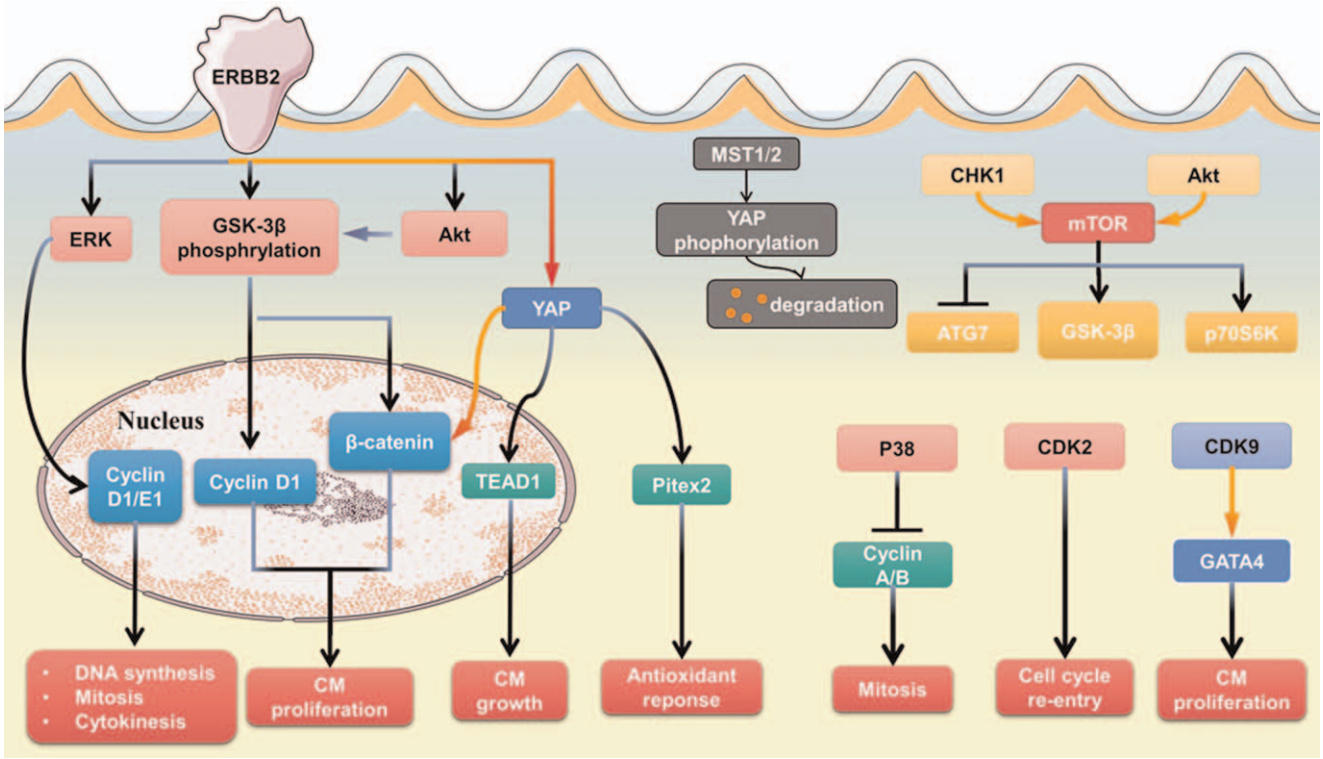

Figure 3: Protein kinases mediated signaling pathways in cardiac regeneration and CM proliferation. AKT: Protein kinase B; ATG7: Autophagy-related gene 7; CDK: Cyclin-dependent kinase; CHK1: Checkpoint kinase 1; CM: cardiomyocyte; ERBB2: Erb-b2receptor tyrosine kinase 2; ERK: Extracellular signal-regulated kinase; GATA4: GATA binding protein 4; GSK: Glycogen synthase kinase; MST: Mammalian Ste20-like kinase; P70S6K: Ribosomal protein S6 kinase; TEAD1: TEA domain family member 1; YAP: Yes-associated protein 1.

foam cells. The relevant regulatory network primarily involves the PI3K-AKT-mTOR pathway, previously discussed in the heart failure section. The functions of macrophage cells in atherosclerosis are regulated by the AKT pathway in two main ways: macrophage polarization and macrophage survival. Macrophages are involved in atherosclerosis in two functional phenotypes: M1 and M2 macrophages. Specifically, M1 macrophages are involved in plaque initiation, progression, and instability, whereas M2 macrophages act reversely.[41,42] On the one hand, AKT activates mTOR in macrophage cells, thereby stimulating histone acetylation and the expression of genes supporting the M2 phenotype.[43] Besides, AKT1 inhibits C/EBPβ to generate the M2 phenotype whereas the deficiency of AKT1 induces M1 cells,[44] which means the balance of AKT isoforms also matters in the network of regulation. On the other hand, recent research has shown that increased macrophage apoptosis significantly accelerates atherosclerosis formation in early and advanced periods.[45] AKT suppresses macrophage apoptosis by phosphorylating apoptosis-regulatory factors Bad and Caspase and activating genes such as murine double minute 2 and IkappaB kinase to support cell survival.[46] Table 1 shows other protein kinases[47] involved in the macrophage function and atherosclerotic plaque formation in addition to AKT pathways.

Myocardial infarction and cardiac regeneration

MI is caused by persistent ischemia and hypoxia of coronary arteries, accompanied by a tremendous amount of myocardial necrosis. Most MI is developed from atherosclerosis of coronary arteries, and the principal treatment of MI has focused on revascularization and reperfusion of blocked arteries. The necrotic CMs, however, are hard to recover. Fortunately, researchers have found that therapies targeting functional molecules are promising,[48] and in this section, we aim to discuss the application of protein kinases in cardiac repair and heart regeneration after MI [Figure 3].

Scientists initially targeted cyclin-dependent kinases (CDKs) family kinases, supposing that they can promote cardiomyocytes' re-entry cell cycle, and they found that CDK2 activation reinitiates cell division in adult CMs and stimulates myocardial regeneration post-injury.[49] Moreover, CDK functions in conjunction with other cell-cycle regulators to promote cell proliferation. For instance, CDK9 acts as a binding partner of GATA binding protein 4, a developmental transcription factor, to regulate the CM proliferation of zebrafish.[50] Another crucial protein kinase that promotes CM proliferation is Erb-b2receptor tyrosine kinase 2, which induces constant cardiomegaly via interactions with proliferation-related pathways such as ERK, AKT, GSK3β/β-catenin,[51] and Hippo/Yes-associated protein 1(YAP) pathway.[52] In the past decade, the importance of ERK, AKT, and GSK3β has been further demonstrated. Regulated by a fetal long non-coding RNA (lncRNA) called endogenous cardiac regeneration-associated regulator (ECRAR), ERK1/2 stimulates DNA synthesis, mitosis, and cytokinesis in both P7 and adult rat CMs.[53] As a consequence of treatment with atorvastatin,

cardiac function improved significantly in rats with the expression of ERK-related proteins increased.[54] AKT, that activated by IL-13, phosphorylates GSK3β at Ser9 to stimulate cyclin-D1 and β-catenin expression, thus promoting CM cell cycle re-entry and endogenous CM proliferation.[10,55] Recently, the use of puerarin, an activator of AKT signaling, has been found to suppress CM apoptosis and reduce MI-induced injury.[56] Similarly, several studies have shown that YAP negatively regulates the WNT signaling pathway and promotes CM proliferation[57] through the interaction with β-catenin on Sox2 and Snai2 genes. YAP also stimulates cardiac regeneration in post-natal mice hearts by binding with Pitx2 and TEA domain family member 1 to strengthen antioxidant response and transcription.[58] What is more, mTOR, a crucial mediator in protein synthesis, cellular growth, and proliferation, also plays an essential role in cardiac regeneration.[14] Mediated by integrin β3, mTOR mitigates autophagy through the regulation of a series of proteins, including autophagy-related gene 7 and interaction with other protein kinases such as GSK-3β to reduce CM death after MI.[59,60] On the other hand, mTOR was also reported to be activated by CHK1 and to initiate CM proliferation in adult rats by activating the ribosomal protein S6 kinase b-1 (p70S6K).[4] Nevertheless, as one of the negative regulators of CM proliferation, p38 downregulates mitosis-related gene expressions such as cyclin A and cyclin B, thus hindering the cell cycle activity.[61] Cardiac-specific p38α knock-out mice show a 92.3% promotion in CM mitoses. Treatment with isoflurane was associated with the observably reduced area of MI, alleviated ischemic damage and inhibited p38 activity.[62]

In addition to targeting CM proliferation after ischemic injury, protein kinases also participate in other cardiac repair strategies post-MI. For instance, overexpression of ROCK increases N-cadherin and integrin ß1expression, thus improving the repair effects of human-induced pluripotent stem cells (hiPSCs)-CM transplantation.[63] In addition, knockdown of integrin-linked kinase (ILK) reduced nuclear factor κB (NF-κB)-related inflammation and restored myocardial repair in exosomes derived from endothelial progenitor cell.[64] Furthermore, Mer TK (Mertk) interacts with signal transducer and activator of transcription (STAT)3 and ERK to accelerate the reparative process, including fibrosis and efferocytosis after MI.[65]

Hypertension and pulmonary arterial hypertension

Hypertension and pulmonary arterial hypertension (PAH) are characterized by high blood pressure and varying degrees of physiological and biochemical changes in the vessel wall, eventually leading to left/right ventricular remodeling. The progression of hypertension and PAH is vitally regulated by the renin-angiotensin system, and recent findings have confirmed the role of protein kinases in these pathways.

In response to angiotensin II (Ang II), the primary effector of RAS, ROCK critically regulates SMC and vascular contraction activity. On the one hand, ROCK suppresses myosin light chain (MLC) phosphatase in SMC, increases Ca^{2+} sensitivity of SMC and promotes MLC phosphorylation, which enhances the interaction between actin and myosin, causing excessive contraction of SMC.[66] On the other hand, ROCK reduces the stability of endothelial nitric oxide synthase mRNA, thus attenuating NO production and the vasodilation function of endothelial cells.[67] In addition, researchers found that gene and pharmacological inhibition of ROCK in the central nervous system of rats can significantly decrease mean blood pressure and urinary norepinephrine excretion,[68] indicating that ROCK works in regulating the sympathetic nervous system tone.

ERK1/2 also regulates SMC contraction and cellular survival. For instance, activating ERK by Ang II increases the level of Ca^{2+} within SMCs and thus triggers SMC excessive contraction.[69] What is more, ERK stimulates the gene expression of c-fos and increases activator protein-1 (AP-1) activity.[70] The transcription factor complex AP-1 is the dimer product of c-Fos and c-Jun, activated by another Ang II-activated protein kinases: c-Jun N-terminal kinase (JNK), ultimately promotes cell differentiation and migration.[70] Similarly, the increased activation of ERK and AKT in hypoxia conditions was reported to promote hypoxia-inducible factor (HIF-1) α expression.[71] HIF-1 α subsequently augments proliferative genes transcription, promotes pulmonary arterial smooth cell proliferation and results in arterial remodeling.[72] Further, AKT enhances protein synthesis and SMC proliferation via the AKT-mTOR-S6K1 pathway. Recent researches have also shown that resveratrol inhibited the proliferation of pulmonary arterial SMCs and right ventricular remodeling by suppressing the ERK and AKT pathways.[73]

Cardiac ischemia/reperfusion injury

The treatment of MI focuses on the early opening of blocked vessels and the reperfusion of ischemic areas. However, reducing infarct size and protecting CM from extra injury during a cardiac ischemia-reperfusion (I/R) episode is of great importance. In this section, we will demonstrate several protein kinases that are core mediators in protecting I/R injury.

Ischemic preconditioning and post-conditioning trigger signal cascade transduction to mitigate reperfusion insult, which for the most part involves the regulation of mitochondrial function. Protein kinases crucially participate in this process via four main mechanisms. First, activated by ischemic post-conditioning, Janus kinase-phosphorylates mitochondrial STAT3 on Tyr705, strengthens interaction with the respiratory chain and reduces ROC production to maintain mitochondrial function.[74] Second, several crucial protein kinases, such as MAPK and casein kinase 1 (CK1)[75] interact with connexin 43 to limit the reperfusion damage via the closure of mitochondrial permeability transition pore.[76] Furthermore, combined with Tribbles homologue 3, AMPK-related protein Snf1-related kinase (SNRK) downregulates uncoupling protein 3 and ameliorates mitochondrial efficiency.[77] The activation of SNRK was associated with maintaining cardiac contractibility and function, decreasing glucose metabolism, and reducing oxygen consumption. Inversely, DNA-dependent protein kinase

catalytic subunit (DNA-PKcs) is a negative regulator of cardiac protection post-I/R. The inhibition of DNA-PKcs reduces the degradation of Bax inhibitor-1, attenuates oxidative stress, mitigates mitochondrial apoptosis, and prevents I/R injury.[78]

In addition to targeting mitochondrial quality control, protein kinases also protect CM from I/R injury in many other practical ways. For instance, using cardiac-specific CaMKII δ knock-out mice, CaMKII δ has been observed to trigger inflammation response after ischemia/reperfusion through activation of NF-κB signaling.[79] Mice deficient in CaMKIIδ in hearts were protected against infarct size expansion, increased apoptosis, and declined cardiac function. Besides, pharmacological inhibition of GSK-3β attenuates I/R injury via phosphorylation of AMPKα, activation of downstream mTORC1, Raptor, and the increased expression of the autophagic marker, microtubule-associated protein 1 light chain 3-II.[80] G protein-coupled receptor kinase 2 [81] and ε-PKC[82] were also related to cardiac protection and reduced infarct size post-I/R.

Hypertrophic cardiomyopathy and dilated cardiomyopathy

Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are the two most common types of cardiomyopathies. They are charactered by ventricular hypertrophy or dilation and cardiac dysfunction and will ultimately result in heart failure. Several risk factors, such as gene mutation, hypertension, and overload stress, are responsible for cardiomyopathies. This section describes how protein kinases play a role in HCM and DCM.

As we mentioned in the heart failure section, CaMKII δ is crucially for the hypertrophic growth of CMs through the function of regulating Ca^{2+} and histone movement.[12,19,22] Another core regulatoris the PI3K-AKT-YAP pathway. The activation of YAP by PI3K/AKT promotes HCM progression, and in turn, activates AKT, ultimately forming a positive feedback loop in the process of cardiac hypertrophy.[83] On the other hand, the transcriptional reprogramming of fetal genes is typical in patients with HCM, and the regulation of these genes is closely related to HDACs. Overexpression of CK2α1 phosphorylates HDAC2 at S394A to stimulate pro-hypertrophic genes transcription[84] whereas pharmacological inhibition of MEK markedly improves clinical and cardiac outcomes in infants with RIT1 mutations-induced HCM.[85] A further observation indicates that MEK1/2 participates in the process of myofibril disarray induced by RAF1 mutations. ERK5, which is crucial to CM enlargement, was also found to be increased during this process.[86]

Increased mitochondrial CaMKII activation was also relevant to left ventricular dilation in mice after MI. RA306, a selective CaMKII inhibitor, has significantly improved cardiac function, including ejection fraction and cardiac output in the model animal with DCM.[87] What is more, ILK, which colocalizes with SERCA2a and β-actinin, acts as a scaffolding protein that binds to the product of PI3K, PI3,4,5-triphosphate and improves the transduction of contractility and modulated CM relaxation in DCM.[88] Similarly, AMPK phosphorylates troponin I and enhances Ca^{2+} sensitivity in CM. Rats with cardiac-specific AMPK β1/β2 knock-out exhibit evidence of DCM and more cardiac function reduction.[89] GSK-3 is another critical mediator of cardiac homeostasis, and when GSK-3 isoforms (GSK-3α/β) were knocked out, mice showed excessive DNA synthesis, multinucleation and notable activation of DNA damage, and cell apoptosis.[90] MAPK pathways are critically involved in lamin A/C gene (LMNA) mutation-related DCM.[91] Treated with ERK and JNK inhibitor, the expression of RNAs encoding sarcomere peptide precursors and proteins required for sarcomere architecture were attenuated with the improvement of ejection fraction and suspension of ventricular dilatation.[92] In 2019, the researchers demonstrated ERK1/2 activation in mice with LMNA mutation-induced DCM phosphorylated formin homology domain-containing proteins (FHOD)1 on S498 and FHOD3, subsequently inhibiting their actin-bundling activity and negatively regulated nuclear movement.[93] These findings may describe the mechanism behind LMNA mutation-caused DCM in part.

Arrhythmia

Arrhythmias are defined as disturbances in the regular rhythm of heartbeats. It can be divided into bradyarrhythmia and tachyarrhythmia. Among them, atrial fibrillation is the most common persistent clinical arrhythmia. An increasing number of arrhythmia pheno-types are affected by the dysfunction of protein kinases signaling.

Tachyarrhythmias, such as atrial fibrillation, are caused mainly by re-entry, abnormal autonomy, and early depolarization or late depolarization. Through the phosphorylation of ryanodine receptor 2 (RyR2) and a variety of membrane voltage-gated channels, including L-type Ca^{2+} channels, voltage-gated Na^{+} channels, and voltage-gated K^{+} channels,[94] CaMK promotes atrial fibrillation. Abnormal activation of membrane voltage-gated ion channels disturbs the ion current during depolarization and repolarization of action potentials (APs), resulting in inhomogeneous AP propagation and repolarization dispersion and causes trigger activity.[95] Meanwhile, the overactivation of RyR2 increases the inward current from the Na^{+}/Ca^{2+} exchanger (NCX) and increases the leak of SR Ca^{2+} as well, particularly during diastole. The increase in NCX is a potential cause of delayed afterdepolarizations, a predisposing factor to AF. Moreover, although the CaMK-dependent phosphorylation of PLN and SERCA2a strengthens the Ca^{2+} recruitment to SR, the increased SR Ca^{2+} leak under pathological conditions remains uncompensated.[96] Recently, studies found that CaMK can be activated by hyperglycemia in addition to the increased reactive oxygen species (ROS) and Ca^{2+}.[97] The O-GlcNAc modification of AMPK enhances SR Ca^{2+}-release, suggesting a potential therapeutic target for diabetes-related AF patients.

AMPK, on the other hand, acts as a protector against the occurrence of AF. AMPK[98] positively regulates membrane ion channels and atrial gap junction proteins such as connexin 40,43,45 to prolong the effective refractory period and reduce AP duration, thus destabilizing the reentry rotors. Second, acting inversely to CaMK, AMPK

reduces diastolic intracellular calcium through the promotion of ATP synthesis, maintenance of the balance between glucose and lipid metabolism, and inhibition of CaMK kinase.[98,99] Additionally, AMPK plays a role in the adaptative remodeling caused by AF and contractile dysfunction. AMPK suppresses mitochondrial ROS and mTOR-related fibrosis and inflammation pathways.[100] Ca^{2+} ion channels activation and Ca^{2+} sensitivity of contractile myofilaments are also promoted by AMPK to maintain atrial contractility.[98,101] Furthermore, AMPK has been reported to be activated by metformin and targets hepatocyte nuclear factor-4[102] to reduce transforming growth factor-β transcription and ERK-mediated profibrotic pathways. These findings indicate that targeting AMPK may lead to clinical translation, and more relevant studies and clinical trials are needed. SPEG and PKA are also important in targeting AF progression. These two kinases regulate RyR2 and SERCA2a and play a role in SR Ca^{2+} release as well.[103,104] In sum, the anti-arrhythmia function of protein kinases is widely studied, and targeted drugs are in development.

As for bradyarrhythmia, the role of protein kinases is also irreplaceable. ROCK was reported to be involved in the developmental process of the atrioventricular node, and the alteration of ROCK expression causes atrioventricular conduction disorders in mice. Embryos treated with ROCK inhibitor Y-27632 exhibited first-, second-, and third-degree atrioventricular block with different degrees of morphological abnormalities,[105] which provides a theoretical basis for further research on the pathophysiology and treatment of atrioventricular block. In addition, AMPK was also found to regulate human intrinsic heart rate. The γ2-AMPK downregulates sinoatrial cell pacemaker to lower heart rate, and the loss of γ2-AMPK will conversely induce the phenotype of increased heart rate, indicating the potential of AMPK in the research of sinus bradycardia and sick sinus syndrome.[106]

Clinical perspective

The modulation of protein kinase activity is an attractive target for drug development and clinical application. A large number of pre-clinical and clinical trials have been conducted, and the results are mixed. Targeting PKC, a study involving 193 patients with chronic heart failure showed that flosequinan significantly improved cardiac function and symptoms compared to placebo.[107] However, flosequinan reportedly increased deaths and hospitalizations in a later study.[108] One more PKCβ inhibitor, rutuxistaurin, improved the cardiac contractility and ejection fraction in a large animal model of HF, representing a new therapeutic approach.[109] ROCK is the critical regulator of SMCs, and highly selective intracoronary injection of ROCK inhibitor fasudil was reported to relieve refractory coronary vasospasms.[110] The following clinical trials evaluating the clinical outcomes of ROCK inhibitor intracoronary injection in MI and atherosclerosis have been in progress (NCT03753269, NCT00120718). The vital function of p38 in the progression of MI has been confirmed. Despite this, the p38 inhibitor losmapimod is not therapeutically effective for treating acute MI.[111] One possibility is that oral p38 inhibitors cannot reach a sufficient concentration in the infarct area and targeted cells. In the field of arrhythmia, one study that included 113 patients with sleep-disordered breathing revealed elevated CaMK-dependent ion channel activity and relevant proarrhythmic activity.[112] Additionally, multiple commonly used clinical drugs, such as metformin and dapagliflozin, have been found to have anti-arrhythmic effects associated with AMPK activation.[113,114] However, although many studies have demonstrated the regulatory network of protein kinases, there are still many challenges to clinical translation and application. One of the urgent problems is the precise delivery of protein kinases to the heart's damaged areas and target cells.

Conclusion

The mechanisms of CVDs and their regulatory network are still not exactly precise. Specific protein kinases have been proven to act as molecular regulators in multiple CVDs in the past two decades. Targeting protein kinases has been effective in triggering endogenous CM proliferation post-MI. The progression of atherosclerosis is also associated with protein kinases. Additionally, several protein kinases, such as Akt and CaMK, participate in more than one abnormal cardiac state and mediate diverse phenotypes through multiple signaling pathways. These findings indicate that targeting these crucial protein kinases may be an efficient choice in CVD diseases.

Although the cardioprotective effects of protein kinases inhibitors or activators have been demonstrated in vitro and *in vivo*, pre-clinical and clinical studies and evidence are still insufficient. Besides, considering the extensive involvement of protein kinases in multiple organs and cell types, the non-targeted application of protein kinases may harm other organs and cells whereas treating CVDs. Even different subtypes of the same kinase may act oppositely. Therefore, further studies are required to reveal how protein kinases interact with other functional proteins and signal pathways. The targeted design of protein kinases will be more important in future clinical applications. In sum, the translational studies of protein kinases are still challenging and promising, and more profound studies are needed to fulfil their potential for therapeutic applications.

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Conflicts of interest

None.

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