



Evolution of β -catenin-independent Wnt–GSK3–mTOR signalling in regulation of energy metabolism in isoproterenol-induced cardiotoxicity model

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Abstract

Objective Isoproterenol (ISO) is widely used agent to study the effects of interventions which could prevent or attenuate the development of myocardial infarction. The elements of pathological events revealed that myocardial infarction requires demand for energy by regulation of cell cycle during isoproterenol-induced cardiac toxicity. Like myocardial infarction, inflammation and fibrosis along with metabolic dysfunction in this model. The classical Wnt/ β -catenin pathway is disrupted during ISO-induced myocardial infarction by hypertrophy and remodeling. However, few studies have reported the role of non-canonical Wnt signalling in cardiac disease.

Method Certain molecules have suggested the inhibition of Wnt could up-regulate key energy sensor and cell growth regulator mTOR. Mechanistic target of rapamycin (mTOR) inhibition of GSK-3 β is a major

Result The GSK-3 β could negatively influence the mTOR activity and produce energy dysfunction during stress or hypoxic condition. This suggests that the inhibition of GSK-3 β by Wnt signalling could up-regulate mTOR levels and thereby restrict energy myocardium, hence energy balance and prevent cardiac toxicity in rodents.

Conclusion We heavily discuss a novel therapeutic role of the β -catenin independent Wnt/GSK-3 β -mTOR axis in attenuation of ISO-induced cardiotoxicity in rodents.

Keywords Isoproterenol · Cardiotoxicity · GSK-3 β · mTOR · Wnt signaling · Myocardial infarction

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Introduction

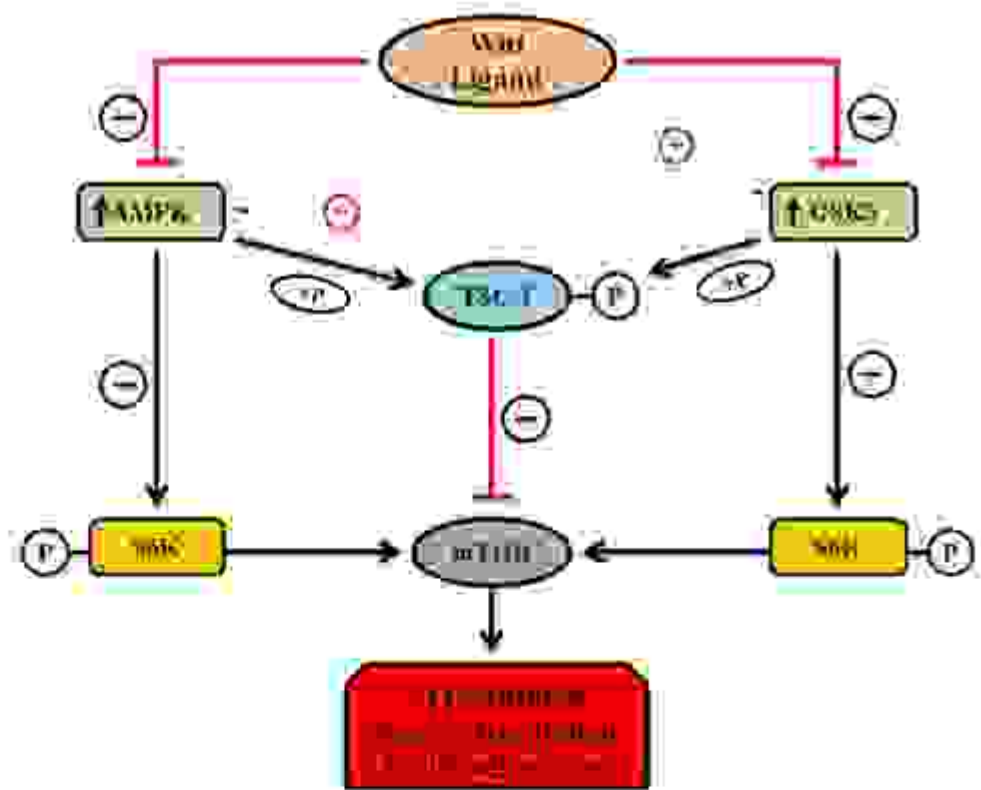
Wnt/Wingless-related integrator (Wnt) signalling mediates diverse pathobiological targets by canonical/non-canonical signalling in certain cardiovascular injury induced by hypercholesterolemia and cardiac arrest syndrome [1]. Wnt signalling by Wnt ligands, β -catenin core protein complex destruction and triggers the pathway to induce transcription of Wnt-specific genes resulting in cell hypertrophy, mitosis and cell proliferation by suppression of GSK-3 β [2]. Moreover, the up-regulation of Wnt/ β -catenin signalling induces fibrosis in epicardial cells isolated from post-infarcted hearts by increasing epithelial mesenchymal transition [3]. The most commonly activated gene regulated by Wnt signalling is WNT1 β -catenin signalling pathway (pathway) [4,5]. Wnt1, β -catenin, TCF, GSK-3 β , APC, Axin, and other proteins form a complex that inhibits β -catenin transcription and causes development and the negative regulator Axin [5]. However, certain conditions have highlighted the potential role of β -catenin independent signalling in particular Wnt-GSK-3 β -Akt-mTOR pathway in the regulation of energy balance and metabolism in cells [6]. The GSK-3 β -glycogen synthase kinase (a serine/threonine kinase, which during insulin induced phosphorylates glycogen synthase and negatively influences the capacity of cells to synthesize and store glycogen [8]). Indeed, the inhibition of GSK-3 β activity was observed to exert cardioprotective in diabetic mice by decreasing anxiety, hyperalgesia, oxidative stress, and lipid accumulation mediated by β -catenin mediated signaling [9]. Akt/PKB (protein kinase B) and PDK1 (phosphoinositide-dependent kinase-1) and PDK2 (phosphoinositide-dependent kinase-2) are also involved in the regulation of energy balance and metabolism in cells [10]. Specifically, GSK-3 β affects energy regulation were reported to be mediated by inhibition of TSC2 (tuberous sclerosis complex 2) and activation of mTOR signaling. Since, TSC2 is a downstream target of GSK-3 β and a negative regulator of mTOR activation, when loss of Rb or p16INK4a promotes inhibition of GSK-3 β resulted in increased proliferation [11]. Clinical improvement of depression and phase II trials mediated by suppression of TSC2 and activation of mTOR-induced translational activity [12]. Importantly, the basal glucose levels were increased upto 3 fold in mice with inhibition of GSK-3 β , thus suggesting a central role of GSK-3 β -TSC2-mTOR axis in regulation of cellular energy balance independent of other signaling.

GSK-3 β is a downstream target of Wnt and by negative feedback mechanism inhibits activation of Wnt signaling. It has been demonstrated that Wnt activation regulates mTOR activity in HEK293T cells through signaling pathway [9]. Cells are known to grow by suppressing GSK-3 β and negative it direct relationship between the Wnt, GSK and mTOR signaling [13]. The canonical activation

of GSK-3 β and AMPK (activated protein kinase) are both reported to inhibit the mTOR activity by decreasing the phosphorylation of S6K (ribosomal protein S6 kinase) and enhancing the phosphorylation of TSC2 protein. The AMPK is a known energy regulator of cellular energy and has been reported to complex with β -catenin and promote its proteasomal degradation, thus which it is used as a proteinase ubiquitin ligase to target β -catenin [14]. These findings suggest that GSK-3 β and AMPK are negatively regulated by Wnt signaling. Indeed, the activation of Wnt receptor by Wnt ligands, activates the GSK-3 β and AMPK mediated inhibitory effects on mTOR activation [15]. The signaling molecule mTOR is recognized as a key regulator of cell growth and cancer immunogenesis [16]. In addition, mTOR activation is responsible for maintaining energy metabolism by enhancing the transcription of peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) and novel regulatory element-binding protein 1 (NREB1) signaling. Interestingly, the activation of mTORC1 by PGC-1 α promotes mitochondrial biogenesis, ATP production and protein metabolism [17]. Specifically, mTORC1 activates β -oxidation pathway for maintaining energy stores by promoting fatty acid oxidation mediated by β -oxidation enzyme activation and increased signaling of fatty acid molecules. Akt is a central mediator of glucose homeostasis [18]. In addition, tyrosine kinase-induced inhibition of cellular ATP synthesis and protein translation is mediated by inactivation of mTORC1 kinase suggesting a direct role of mTOR in cell growth and energy expenditure [17, 19]. Overall, these evidence imply that regulation of mTOR level by Wnt activation through GSK-3 β inhibition is critical for regulating cellular energy metabolism during post-infarction. In addition, the strong link between Wnt-GSK-3 β and mTOR signaling is demonstrated (Fig. 1).

In particular, mTOR receptor agents, which are currently not clear, is used by various experimental myocardial infarction models. Tyrosine kinase inhibition results in increased myocardial infarction work with concomitant increase in oxygen demand and reduced supply result in inflammatory stress, cardiac damage and fibrosis [20]. Therefore, pathological changes including increased oxidative stress, inflammation, calcium overloading and mitochondrial dysfunction, which lead to cardiac dysfunction, fibrosis and necrosis [20]. Accumulating evidence has shown that downregulation of energy metabolism and mitochondrial function are initial pathological events involved in the development of experimental induced cardiac infarction. Indeed, a decline in ATP synthesis, activation of oxidative stress and mitochondrial oxygen consumption was observed during early post-myocardial infarction. O $_2$ in period in post-infarction model [21]. Another study demonstrated that administration of hydrocortisone in 3 days

Fig. 1. Wnt/PCP and AMPK contribute to energy homeostasis. Wnt/PCP signaling activates the phosphorylation of TSC-2 and reduces phosphorylation of S6K which is responsible for expression of mTOR signaling. Simultaneously, Wnt/PCP induces mTOR activation by activating the phosphorylation of mTORC2 and S6K.

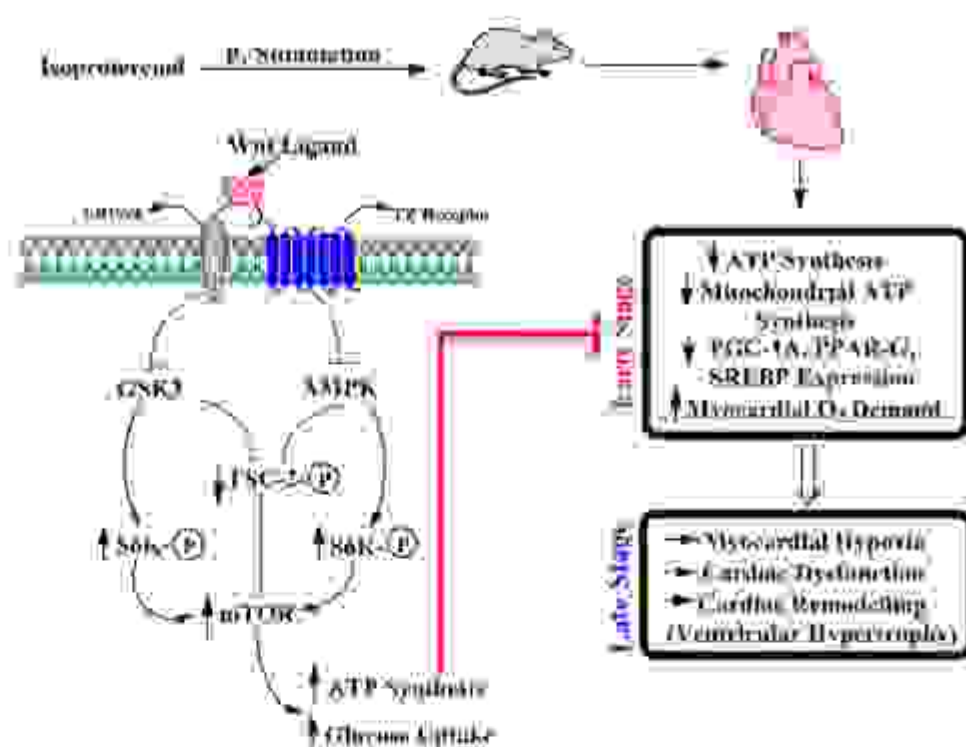


kg body weight for 7 days lead to reduction in tail pressure relaxation, phosphorylation of phospho-tyrosine in the tail, and a decrease in the tail length. In addition, it may post-translational modification of cardiac workload and associated with normal myocardial blood flow depicting a functional link between hyperbaric and supply. Biochemical activation of skeletal muscle from hyperbaric oxygenated and hypoxic conditions, such as hypertrophy. Through both of these routes, decreased partial oxygen pressure, increased oxygen, and hypoxia, mTORC1 signaling was clearly visible in the tail. Thus, it is clear that myocardial hypertrophy induced by hyperbaric oxygen in normoxic state of energy imbalance and mitochondrial dysfunction which triggers various pathological cascades leading to cardiac overgrowth and remodeling.

Wnt signaling protein has been observed to be directly implicated in mediating H₂O₂-induced stimulation-induced hyperphosphorylation of AMPK. In a study, inhibition of Wnt signaling complex subunit dishevelled (Dvl) resulted in induction of hypertrophic changes in embryonic myocytes exposed to hyperbaric oxygen. This suggests that the activation of canonical Wnt/PCP pathway is essential for promoting hyperbaric-induced cardiac structural adaptation [2]. However, as discussed above, the non-β-catenin-dependent Wnt signaling pathway is involved in regulation of cellular energy status

by suppressing GSK-3-mediated inactivation of Akt and mTOR signaling. In this work, measuring the tail hypertrophy demonstration revealed an increase in chemical energy charge and creatine phosphate/creatine ratio (an indicator of reduced ATP availability) in heart at an early period of 3 h. This suggests that cardiac energy imbalance and myocardial dysfunction are early pathophysiological events which might result in cardiac dysfunction and structural changes at later stages of hyperbaric oxygenation. The same interventions which sustain the cellular ATP levels by improving mitochondrial function will play a role in the development of cardiac functional and structural damage in hyperbaric oxygenated mice. We have previously shown Wnt β-catenin-independent mechanism whose activation could be important for regulation of myocardial ATP levels in early stage of hyperbaric-induced cardiac hypertrophy. Importantly, the Wnt-mediated GSK-3 inhibition will stimulate low energy sensor and regulator molecule mTOR by involving Akt signaling. This process may be ATP levels in hyperbaric oxygenated animals prevent the development of cardiac dysfunction and structural changes. In hyperbaric oxygenated animals, Wnt inhibition of Wnt/PCP signaling is important for preventing cellular energy status remodeling, maintaining Wnt/PCP and GSK-3 inhibition and activation of mTOR signaling will play a role in maintaining energy levels during the early period of ISO model by controlling the oxygen and energy

Fig. 7 Regulation of myocardial ATP and energy metabolism. Mitochondrial dysfunction is early phase of the disease. The pathogenesis includes cardiac hypertrophy and energy starvation of β -adrenergic stimulation. Wnt signaling attenuates energy and oxygen consumption by targeting GSK-3 β -mTOR pathway. This may cause and prevent compensatory up-regulation of the state.



adrenergic stimuli, we discussed a new therapeutic role of our synthetic Wnt/GSK-3-mTOR axis in β SO-induced myocardial hypertrophy model. We propose that the early and early intermediate Wnt/GSK-3-mTOR axis could exert cardioprotective effects in ISO model by sustaining intracellular basal energy levels and thereby preventing mitochondrial dysfunction and histological changes. Further experiments are required to validate the therapeutic effects of activating Wnt-GSK-3-mTOR axis in hyperadrenergic-induced cardiovascular system. If this hypothesis is validated experimentally, we might offer a new therapeutic treatment for the therapy prevention of non- β -adrenergic dependent Wnt-GSK-3-mTOR axis in cardiac disease associated with hyperadrenergic conditions. Effect of Wnt-GSK-3-mTOR axis on energy dysregulation-induced by ISO administration is presented in Fig. 7. This study discusses the possible cardioprotective role of Wnt-GSK-3-mTOR axis in preventing early pathological changes in hemodynamic heart failure model. However, from the temporal assessment of changes in this study during pathologic condition by the increased energy level, it is likely that this study needs to be advanced. Therefore, it would be important to study the temporal changes in the energy level in various pathological conditions including energy dysregulation, cardiac hypertrophy and failure underlying high-dose β -adrenergic stimulation.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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