The Role of RP105 in Cardiovascular Disease through Regulating TLR4 and PI3K Signaling Pathways*

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Summary: Raidoprotective 105 (RP105) was first discovered on the surface of mouse B cells and it has been demonstrated that RP105 can function as an inflammatory regulator in cardiovascular disease (CVD), such as myocardial ischemic reperfusion injury (MI/RI), atherosclerosis and myocardial infarction (MI). As a member of Toll-like receptor (TLR) homolog which is capable of regulating toll-like receptor (TLR4) signaling pathway, RP105 is implicated in various biological processes. Mounting evidence suggests that RP105 regulates the function of TLR4 and phosphoinositide 3-kinase (PI3K) signaling pathways. Here, we review the effect of RP105 on CVD through regulating TLR4/PI3K signaling pathways.
Key words: RP105; TLR4 signaling; PI3K signaling; cardiovascular disease

It is well known that raidoprotective 105 (RP105) is a member of toll-like receptor (TLR) family which has 22 leucine-rich repeats (LRRs) in its extracellular domain and lacks the intracellular toll interleukin receptor (TIR) domain. RP105 was first discovered on mouse B cells surface implicated in B cell activation[1], and it has the ability to promote cellular proliferation and to enhance B cell dependent inflammatory process[2]. TLR4 is tightly associated with its adaptor molecule MD-2 to recognize bacterial lipopolysaccharide (LPS) on the cell surface[3] and it presents on circulating cells and cardiomyocytes. However, generous researches revealed that RP105-MD1 can downregulate LPS response by the way of interaction with TLR4-MD2[4]. Additionally, recent researches have identified that PI3K signaling pathway has a close relationship with RP105 in alleviating myocyte apoptosis and thereby ameliorating myocardial damage in myocardial ischemic reperfusion injury (MI/RI)[5]. So far, researches have discovered that complicated signaling pathways were involved in MI/RI. Recently, studies have detected that RP105 can play a potential therapeutic role in cardiovascular disease (CVD) through TLR4 and PI3K signaling pathways.

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*This study was supported by the National Natural Science Foundation of China (No. 81770360 and No. 81800258).

1 Biological Characteristics of RP105

RP105 is an important accessory molecule acting as a regulator of TLR4 signaling, and TLR4 signaling is dependent on an extracellular associated accessory protein, MD-2. Surface expression and activation of RP105 are similarly dependent on MD-2 homologue, MD-1 (fig. 1). In fact, RP105 expression mirrors that of TLR4 on myeloid cells, including monocytes, macrophages, and dendritic cells (DC)[6]. Unlike myeloid cells, B cells do not express MD-2, thus representing an opposite role in TLR4 signaling[7]. TLR4, expressed on the cell membrane of monocytes

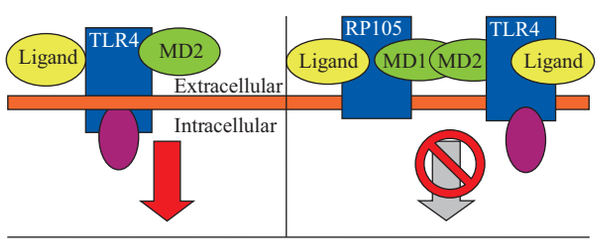

Fig. 1 The role model of TLR4 and RP105
Activation of TLR4-MD2 by ligand binding can activate intracellular signaling pathways and initiate a downstream signaling cascades (left). RP105-MD1 can bind to TLR4-MD2 and alter TLR4 signaling cascades, but RP105 has no signaling capacity as it lacks the intracellular toll interleukin receptor (TIR) domain.

and macrophages, can promote NF-κB-mediated inflammatory gene transcription and the release of inflammatory cytokines, for example, pro-arteriogenic TNF-α[8], and it has been proved that RP105 can act as a specific inhibitor of the TLR4-mediated inflammatory response. Recently, RP105 has been found to be related with PI3K/Akt signaling pathway.

1.1 RP105/TLR4 Signaling

Yang *et al* demonstrated that overexpression of RP105 significantly reduces the level of TLR4, and attenuates the phosphorylation of P38MAPK and transcriptional factor c-Jun/activator protein-1(AP-1)[9]. P38MAPK aggravates myocardial apoptosis in its phosphorylated state[10], and the activation of AP-1 is needed for P38MAPK expression[11]. AP-1 belongs to c-Jun and c-Fos protein families with the function of inducing apoptosis of cardiac cells which is regulated by MAPK. Yang *et al* found that MI/RI myocardium apoptosis was aggravated when the levels of TLR4, P38MAPK, and AP-1 were up-regulated. On the contrary, overexpression of RP105 down-regulates the levels of TLR4, P38MAPK and AP-1 and then alleviates myocardium apoptosis. This suggests that RP105/TLR4/P38MAPK/AP-1 signaling pathways are involved in the process of cardioprotection, and could be regarded as a therapeutic target for CVD.

TLR4/myeloid differentiation factor 88 (MyD88) and TLR4/TIR domain containing adapter including interferon β (TRIF) signaling pathways seem to be another cardiac protective mechanism of RP105 in heart[12]. As such, RP105/TLR4/MyD88/TIR signaling pathways can be regarded as another therapeutic target. Additionally, the TLR4/NF-κB signaling pathway can be inhibited by RP105, and function in a cardiac protective role. MyD88 and NF-κB are both downstream factors of TLR4 which can be inhibited by RP105. RP105 is an important accessory molecule acting as a regulator of TLR signaling[13], it functions as an inhibitor of TLR4 signaling in myeloid cells such as macrophages, while a promoter in B cells. Yang *et al* found that the levels of TLR4 protein and mRNA were elevated in MI/RI, along with the increase of TNF-α and IL-6[14], which may be due to the inhibition of RP105 expression.

1.2 RP105/PI3K Signaling

As a unique member of TLR family, the type I transmembrane protein RP105 was originally found on the surface of B cells functioning as a mediator of activation and proliferation and was later discovered in PI3K signaling pathway either. On the other hand, PI3K and its downstream Akt function as a conserved family of signal transduction enzymes which are involved in modulating inflammatory cascades, apoptotic and autophagic processes caused by MI/RI[15]. Ha *et al* have also proved that stimulation of PI3K/Akt signaling pathway alleviated myocyte apoptosis and thereby alleviate MI/RI[5]. As such, there is generous encouraging evidence showing that PI3K/Akt signaling pathway is a promising therapeutic target for alleviating MI/RI that happened independent of stereotypical TLR4 signaling pathway. Thus, RP105 can function in both ways to alleviate CVD.

2 RP105 and CVD

The research of RP105 is becoming more and more prevalent nowadays, and it can function as a signaling pathway regulator in CVD, such as MI/RI, atherosclerosis and MI. P38MAPK, AP-1, MyD88 and NF-κB are downstream signaling molecules of TLR4. RP105 can inhibit the expression of TLR4, thus alleviate myocardium apoptosis and inhibit inflammatory through TLR4/P38MAPK/AP-1, TLR4/MyD88, TLR4/NF-κB pathways. Moreover, RP105 can alleviate symptoms of CVD patients by inhibiting inflammation and mitigating cardiomyocyte apoptosis and autophagy through stimulating PI3K/Akt pathway.

2.1 RP105 and MI/RI

To testify whether RP105 has an impact on MI/RI, Yang *et al*[9] demonstrated that overexpression of RP105 significantly inhibited the level of the pro-apoptotic protein, such as Bax, casepase-3, cytochrome c, and increased the anti-apoptotic protein Bcl-2. The research indicates that RP105 therapy via adenovirus vectors is a promising cardioprotective method to elicit anti-apoptotic effects in MI/RI[9]. RP105 deficiency would lead to an unrestrained TLR4-mediated inflammatory response and hence to hinder blood flow recovery after myocardium ischemia. As we have known, RP105 is a negative regulator of TLR4 response. Bastiaansen *et al* did an experiment using RP105-/- and wild type (WT) mice to investigate the contribution of RP105 to post-ischemic neovascularization in a hind limb ischemia (HLI) model, it was found that the level of pro-inflammatory NF-κB which can mediate gene transcription increased in RP105-/- mice. Moreover, impaired recruitment of Ly6Chi can result in impaired arteriogenesis. As a consequence, RP105 deficiency can result in enhanced inflammation[16].

As it is known to all, the most viable therapeutic approach for coronary heart disease is to re-establish blood supply for ischemic myocardial tissue[17], at the same time, the way of coronary revascularization to re-establish blood supply can result in myocyte damage which is MI/RI. It has been proved that TLR4 increased significantly in MI/RI[18] and *in vitro* studies showed that the activation of TLR4 can lead to the mitochondria-associated apoptosis cascades. The TLR4-dependent P38MAPK and AP-1 signaling pathways play a significant role in MI/RI[19]. It has been proved that blocking the apoptotic pathway may exert a protective effect against MI/RI[20, 21]. What's more, RP105 is

strongly related with the inflammatory response in MI/RI[22]. RP105 can not only reduce the expression of TLR4, but also attenuates the phosphorylation of P38MAPK and transcriptional factor c-Jun/AP-1. Additionally, PI3K/Akt signaling pathway has also been proved to be a therapeutic approach in MI/RI. However, the regulation of TLR4, P38MAPK, AP-1, and PI3K/Akt in the myocardium following MI/RI is rarely known.

2.2 RP105 and Atherosclerosis

Atherosclerosis in coronary artery is the major cause of cardiovascular events and complications worldwide[23]. It is characterized by the leukocytes infiltrating into the intima and causes a local inflammation in vessel[24]. Moreover, inflammation plays a significant role in the initiation, growth, rupture of atherosclerotic plague[24–26]. It has been proved that different kinds of leukocytes are linked to atherosclerotic lesion formation, including macrophages, T cells, mast cells, B cells and so on[27]. TLR function is linked to tissue macrophages or foam cells. What's more, TLR4 deficiency or its downstream adaptor protein Myd88 has been proved to reduce atherosclerosis formation[28]. It was found that deficiency of TLR4 in atherosclerosis-prone mice would develop smaller atherosclerotic lesions[29] and also TLR4 antagonist could reduce early plague formation[30].

To testify the role of RP105 on circulating cells in atherosclerosis plague formation, Karper *et al*[2] did an experiment with irradiated low density lipoprotein receptor deficient mice and let them receive RP105-/- or wild-type bone marrow. Interestingly, the RP105-/- group displayed 57% reduced plague burden as compared with the wild group and activated B cells were significantly decreased, lesional macrophages were decreased by 40% in RP105-/- mice. It has been proved that RP105 deficiency can result in increased neointima formation through enhancing proliferation of smooth muscle cells[31]. RP105 modulates B-cell function through cell surface receptor TLR4, and proliferation was reduced in RP105 deficiency B cells. A recent article suggested that the expression of B-cell activating factor (BAFF) increases in RP105-/- mice[32], indicating that alterations on proinflammatory B cells can decrease atherosclerotic lesion formation.

To testify the hypothesis, Wezel *et al*[33] did another experiment with low density lipoprotein receptor deficient (LDLr-/-) and LDLr/RP105 double knockout (LDLr-/-RP105-/-) mice, showing that RP105 deficiency reduced plaque size by 40% and deceased lesional macrophages and peritoneal B cells. Plasma lgE and lgM levels are also reduced dramatically. They also found a significant upregulation of RP105 expression in early atherosclerosis which can play a compensatory role. This experiment provided a novel mechanism via which RP105 deficiency may attenuate atherosclerosis.

2.3 RP105 and MI

Myocardial infarction (MI) is the leading cause of death in CVD, the incidence and prevalence of MI resulting in death are increasing nowadays[34]. TLR4 acts as a receptor of innate immune system and has disadvantageous effects on cardiac function after MI. TLR4 is the most studied type of TLRs and plays an important role in MI healing[35]. RP105, an inhibitor of TLR4, can dampen the inflammatory responses induced by the TLR4 activation.

TLR4 and its ligand fibronectin-EDA can result in deteriorating effects on cardiac remodeling after MI[36, 37]. To testify the hypothesis that RP105 deficiency can amplify TLR4 signaling and aggravate cardiac dysfunction after MI, Louwe *et al*[38] did an experiment in RP105-/- or wild-type mice, the inflammatory response increased in RP105-/- group after stimulation with LPS, while, RP105 deficiency did not influence myocardial infract size. What's more, RP105 deficiency could promote cardiac dilatation but didn't affect baseline cardiac function and infract size in MI patients. RP105 can be a theraputic strategy for MI.

3 Conclusions and Perspectives

In conclusion, RP105 has the ability to regulate TLR4/PI3K signaling pathways, it can alleviate MI/RI and MI, but aggravate atherosclerosis, demonstrating that RP105 plays an important role in CVD. Nowadays, RP105 is considered as a promising clinical tool and also a therapeutic target in CVD. To figure out the molecule mechanism clearly and obtain knowledge of RP105 relevant therapeutic medicine for CVD, further studies are required to ascertain the different function of RP105- related signaling pathways in different diseases.

Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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(Received Jan. 20, 2018; revised Dec. 6, 2018)