

# Double stranded RNA dependent protein kinase (PKR) and type 2 diabetes

## Abstract

Type 2 diabetes greatly increases the risk for developing cardiovascular and metabolic disorders. Despite recent development in medical science, scientific understandings on the root mechanisms of type 2 diabetes are still not fully understood, and such insufficient understanding contributes to the relative lack of effective treatments for such diseases. Protein Kinase R (PKR) is a serine threonine kinase activated during various stress conditions. Activation of PKR can increase reactive oxygen species generation, inflammation and induce oxidative stress. In this review we discuss the potential role of PKR in type 2 diabetes, pathways activated by it and the interrelationship between pathways activated. Specific and effective inhibitors of PKR are being developed and can become potential treatment for type 2 diabetes and prevent many diseases.

**Keywords:** PKR, type 2 diabetes, inflammation, insulin resistance

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**Abbreviations:** PKR, protein kinase r; ER, endoplasmic reticulum; Nfkb, nuclear factor kappa-light-chain-enhancer of activated b cells; IKK, inhibitory  $\beta$  kinase; JNK, c-jun n-terminal kinases; Eif-2 $\alpha$ , eukaryotic initiation factor  $\alpha$

## Introduction

Increasing type 2 diabetes and the associated hypertension and cardiovascular diseases in children and adults are major health issues globally with limited treatment options. The explosive increase in type 2 diabetes in the past 2-3 decades has been attributed to high dietary carbohydrates combined with a sedentary lifestyle.<sup>1,2</sup> Such pathological conditions invoke stress related response in the body, one of which is activation of Protein Kinase R (PKR). RNA activated/dependent protein kinase (PKR) is a serine threonine kinase that can directly couple to the metabolic pathway due to its catalytic activity and has a role in pathogen recognition.<sup>2</sup> PKR is activated by a number of signals, such as interferons, viral infection, high cholesterol diet,<sup>3</sup> cytokines, pathogens, irradiation, heme limitation<sup>4-10</sup> endoplasmic reticulum (ER) stress as well as mechanical stress.<sup>11</sup> PKR contains two dsRNA binding domains, one at its N-terminal and the other at its C-terminal.<sup>4,5,12-14</sup> In the presence of a pathogen, the double stranded RNA binds to the N-terminal of the PKR enzyme and leads to the phosphorylation of eukaryotic initiation factor (eIF2A). It activates eIF2AK2 (eukaryotic translation initiation factor 2-alpha kinase 2) which is coded by the eIF2AK2 gene.<sup>2,15</sup> This increases phosphorylation of eIF2 and provide the kinase enzyme better access to its substrate.<sup>13,16</sup> Increase in eIF2 $\alpha$  leads to the inhibition of translation thereby impeding further replication of the virus. In normal state, eIF2 combines with methionyl t-RNA and GTP, which is followed by its combination with the 40S ribosomal subunit. This complex recognizes the start codon during translation. When the larger subunit is additionally combining with this complex, the GTP-eIF2 complex is hydrolyzed to a GDP complex. During an infection, once eIF2 is phosphorylated to its eIF2 $\alpha$  form, the conversion of GTP-eIF2 complex to GDP is inhibited. This results in blocking translation due to low GDP levels and thus prevents viral replication in cells.<sup>17,18</sup>

Type 2 diabetes is associated with elevated blood glucose levels, which in turn will affect plasma insulin levels. Components of the immune system are also changed in type 2 with the most apparent changes occurring in insulin sensitive tissues such as adipose tissue, liver, pancreatic islets as well as the vasculature and circulating leukocytes which in turn may lead to inflammation, insulin resistance, reduced insulin secretion, pancreatic  $\beta$ -cell apoptosis and tissue fibrosis.<sup>19</sup> PKR is also implicated in inflammation and immune dysfunction through its regulation of mitogen-activated protein kinases, interferon regulatory factor 3, nuclear factor  $\kappa$ B and apoptosis.<sup>20</sup>

## Discussion

It has been reported earlier that in PKR knockout (Pkr<sup>-/-</sup>) mice, fasting plasma glucose is reduced while insulin action and insulin-induced Akt phosphorylation is improved as compared to wild type control (Pkr<sup>+/+</sup>) mice. PKR is known to phosphorylate the regulatory subunit of PP2A, which then activates the catalytic subunit of PP2A inducing its phosphatase activity.<sup>3,21</sup> Mice islet  $\beta$ -cells and insulinoma cell lines exposed to high glucose and proinflammatory cytokines showed significantly increased PKR activity associated with significantly inhibited cell proliferation by arresting cell cycle at G1 phase. PKR activation abolished the pro-proliferative effects of IGF-I by activating JNK and disrupting IRS1/PI3K/Akt signaling pathway.<sup>22</sup> Inhibition of PKR reduces stress-induced JNK activation and IRS1 serine phosphorylation *in vitro* and *in vivo*. It has also been reported that treatment with PKR inhibitors, imoxin and 2-aminopurine reduced adipose tissue inflammation, improved insulin sensitivity, and improved glucose intolerance in obese (ob/ob) mice following the establishment of obesity and insulin resistance.<sup>3</sup> PKR is known to directly target and modify the insulin receptor and thus inhibiting insulin action. It has been reported earlier that PKR induces the inhibitory phosphorylation of IRS at site Ser312 and activates the transcription factor, Foxo1, which in turn up-regulates the protein expression level of IRS2. Knockout of PKR (Pkr<sup>-/-</sup>) in mice showed protection against insulin resistance and diabetes.<sup>3</sup>

Under stress condition, JNK negatively controls insulin signaling through serine phosphorylation of IRS1 instead of the normal tyrosine phosphorylation. JNK activation by PKR may also lead to serine phosphorylation of IRS1.<sup>23,24</sup> Nakamura et al.<sup>3</sup> tested this theory by taking Pkr knockout (Pkr<sup>-/-</sup>) and wild type (Pkr<sup>+/+</sup>) primary mouse embryonic fibroblast cells (MEFs) and exposing them to palmitic acid and thapsigargin. In Wild type (Pkr<sup>+/+</sup>) MEFs phosphorylation of IRS1 was observed whereas in PKR knockout (Pkr<sup>-/-</sup>) MEF's, no IRS1 phosphorylation was observed. This proves that PKR is involved in the eventual phosphorylation of IRS1.<sup>3</sup> Garcia et al.<sup>25</sup> reported that when treating wild type (Pkr<sup>+/+</sup>) and PKR knockout (Pkr<sup>-/-</sup>) MEFs with polyinosinic-polycytidylic (PolyI.C), a direct activator of PKR, IRS1 phosphorylation was only observed in wild type (Pkr<sup>+/+</sup>) MEFs whereas no phosphorylation was observed in PKR knockout (Pkr<sup>-/-</sup>) MEFs.<sup>25</sup>

Einarson et al.<sup>26</sup> confirmed the interaction between PKR and IRS1 by Pull down assays, when a direct interaction was observed between IRS1 and PKR.<sup>26</sup> Nakamura et al.<sup>3</sup> reported that PKR causes the direct phosphorylation of the serine 307 residue of IRS1, using TNF- $\alpha$  (known to activate PKR) and TG on both wild type (Pkr<sup>+/+</sup>) and PKR knockout (Pkr<sup>-/-</sup>) MEFs and demonstrated the extent of IRS1 phosphorylation using a phospho specific antibody. It was found that the WT MEFs were able to show the excessive phosphorylation, unlike their counterparts.<sup>3</sup> PKR plays an important role in insulin resistance as well. Nakamura et al.<sup>3</sup> exposed wild type (Pkr<sup>+/+</sup>) and PKR knockout (Pkr<sup>-/-</sup>) mice to high fat diet and observed an increase in insulin induced Akt phosphorylation (Serine 473) in liver and adipose tissue of PKR knockout (Pkr<sup>-/-</sup>) mice as compared to wild type (Pkr<sup>+/+</sup>) control mice.<sup>3</sup> It has been reported previously that direct interactions of PKR occurs with the IKK $\beta$ -NF- $\kappa$ B pathway.<sup>27</sup> PKR directly interacts with and modulates IRS, a critical molecule in insulin action, and has a major regulatory role over JNK activation.<sup>24</sup> Thus pharmacologically targeting PKR may be an effective therapeutic strategy for the treatment of type 2 diabetes. The main limitation we have in this area is that the role of PKR in type 2 diabetes is still in the initial stages of investigation and more and more reports are coming out. Even then, these reports deal with the conditions obesity and diabetes as single entities and describe limited findings on the effects of PKR change in cultured cells and animal models. Apparently, it will take some time before an integrated picture of the role of PKR in the metabolic and cardiovascular disorders starts emerging.

## Conclusion and future prospects

The present review indicates that PKR plays a crucial role in the pathogenesis of type 2 diabetes. Many important questions still remain to be addressed. Current understanding on the inflammatory mechanisms of metabolic syndrome and related disorders are still in its primitive stage. We anticipate in near future eventually these findings will be translated into novel and effective treatments/preventions against diabetes and related diseases.

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## Conflict of interest

Author declares that there is no conflict of interest.

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