Nuclear Factor Erythroid 2 Activation Mediated by PRKCA in Increasing Ca^{2+} Intracellular in Diabetic Condition

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Abstract: *Diabetes increases in the worldwide by prevalence and numbers of complications related to it. The most common risk factor for foot ulcers in diabetic is peripheral neuropathy. In diabetic condition, Ca^{2+} concentration intracellular increases. NRF2 (Nuclear Factor Erythroid 2) acts as a bridging link in various inflammatory and apoptotic pathways impacting progress of diabetic neuropathy. The aim of this study is to predict the pathway of NRF2 activation that is mediated by the increased of intracellular Ca^{2+}. Data interaction between Ca^{2+} and NRF2 were retrieved from STITCH (Search Tool for Interacting Chemicals) which were experimentally and prediction, then were analyzed computationally. Pathway analysis used Cytoscape software. The functional analysis was evaluated using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database. The consequence of increased intracellular Ca^{2+} content is the increases of oxidative stress, ROS (Reactive Oxygen Species) and the translocation of the protein kinase Cα (PKRCA) to the plasma membrane as the initial step of their activations. This study reveals that activation NRF2 by Ca^{2+} intracellular which increases in diabetic condition through PRKCA, PRKCA phosphorylate NRF2 in its Neh2 domain at Ser-40.*

Keywords*: Nuclear factor erythroid 2, Ca^{2+}, PRKCA, Diabetes, Neuropathy.*

Introduction

The prevalence rates of obesity and type 2 diabetes have increased during the last 30 years [3]. The latest global estimate from the International Diabetes Federation (IDF) is that in 2015 were 415 million people with diabetes mellitus and by 2040 the number will be 642 million [16, 31]. Increased numbers of complications related to diabetes, the prevalence of diabetic peripheral neuropathy was 7% in youth with type 1 diabetes and 22% in youth with type 2 diabetes [11].

The most common risk factor for foot ulcers in diabetic contributing to higher than 80% of these ulcers is peripheral neuropathy [1, 27].

In diabetic neuropathy, has been associated with aberrant Ca^{2+} channel expression and function [14, 20]. The divalent Ca^{2+} is one of the most widely utilized second messengers in cellular signaling and one of the repertoires of the cells [7]. Ca^{2+} channels can be irregulated in a diabetic condition, leading to an enhanced calcium influx in neurons [23]. These abnormalities manifest as an increased resting intracellular Ca^{2+} concentration ($[Ca^{2+}]$), decreased activities of Ca^{2+} transporters and decreased stimulus-evoked Ca^{2+} signaling [20]. Ca^{2+} influx increases in cytoplasmic, mitochondria and ER, causing changes in mitochondrial pH and ROS production [8].

Consequence of increased intracellular Ca^{2+} content is the translocation of the protein kinase Cα (PKRCA) to the plasma membrane as the initial step of their activations. Therefore, phosphorylation mediated by the PKRCA is a great importance for intracellular signal transduction [28]. Role of the PKC (protein kinase C) pathway in the ARE (antioxidant response element)-mediated gene expression, and has been suggested that PKC-directed phosphorylation of NRF2 may be a critical event for the nuclear translocation of this transcription factor in response to oxidative stress [9, 13]. High Ca^{2+} accumulation in the cytosol gave a manifestation in stress oxidative. One of protein that takes apart in response to oxidative stress is NRF2. NRF2 acts as a bridging link in various inflammatory and apoptotic pathways impacting progress of diabetic neuropathy [12]. Strategies utilizing a more targeted approach such as focusing on NRF2 (a transcription factor modulating oxidative stress) may provide an enthralling avenue to optimize neuroprotection in diabetes and diabetic neuropathy [18]. NRF2 activation is the key role of recovering from the oxidative stress.

Recently the role of Ca^{2+} to activate the NRF2 is still controversial. The mechanism's detail of Ca^{2+} affects to stress oxidative response will reveal the relation between Ca^{2+} intracellular concentrations in diabetic condition with recovery condition mediated by NRF2. The aim of is study is to predict the pathway of NRF2 activation that mediated by increasing intracellular Ca^{2+}.

Materials and methods

Data retrieval

Protein and calcium interaction data were retrieved from STITCH database. The data of chemicals were linked to another chemicals and proteins by evidence derived from experiments, databases and the literature. Retreived data were interaction network originated from cellular reaction of human [26]. The interaction between biomolecule is essential part in biosystem analysis. The interaction data from STITCH were combined from experimental and prediction data. The researchers chose the high confidence interaction to explore the connection of Ca^{2+} and NRF2 computationally, data were collected in tsv format.

Pathway analysis

The molecular interaction network data from STITCH database were analyzed using CytoScape software v3.5.1. [24]. This software handled basic features such as network layout and mapping of data attributes to visual display properties. It also could combine several pathways and analyses the main role of protein the network. Two interaction data that are (1) calcium interaction with the cytosolic protein and (2) NRF2 interaction with cytosolic protein. Both networks were merged manually by opening the interaction network data and analyzing

the overlap data based on protein name. The merged network data were identified as the main pathway that connect Ca^{2+} and NRF2.

Functional analysis

The functional analysis was evaluated using STRING database [22, 25]. This database provides the proteins functional analysis from numerous sources. The functional data were extracted based on low false discovery rate score (FDR). The functional data were done by selecting "Analyze button" on sting-db web service (https://string-db.org). The data were ranked based on FDR score. The lowest FDR score was choose for reliable functional information. The data were clustered to be three categories that are:

(1) biological process;
(2) molecular function; and
(3) cellular location.

Results and discussion

Ca^{2+} interaction and binding partners

High Ca^{2+} intracellular concentration could affect the the binding partners. This ion could bind several proteins and induce some biological processes. The proteins that have direct interaction to calcium ion are TNC (tenascin C), MYLK (myosin light chain kinase), ADCY1 (adenylate cyclase 1), NOS2 (nitric oxide synthase 2), PRKCA, CAMK4 (calcium/calmodulin dependent protein kinase IV), PLCG1 (phospholipase C gamma 1), CBL (casitas B-lineage lymphoma), EGFR (epidermal growth factor receptor), and PDGFRB (platelet-derived growth factor receptor beta precursor) (Fig. 1).

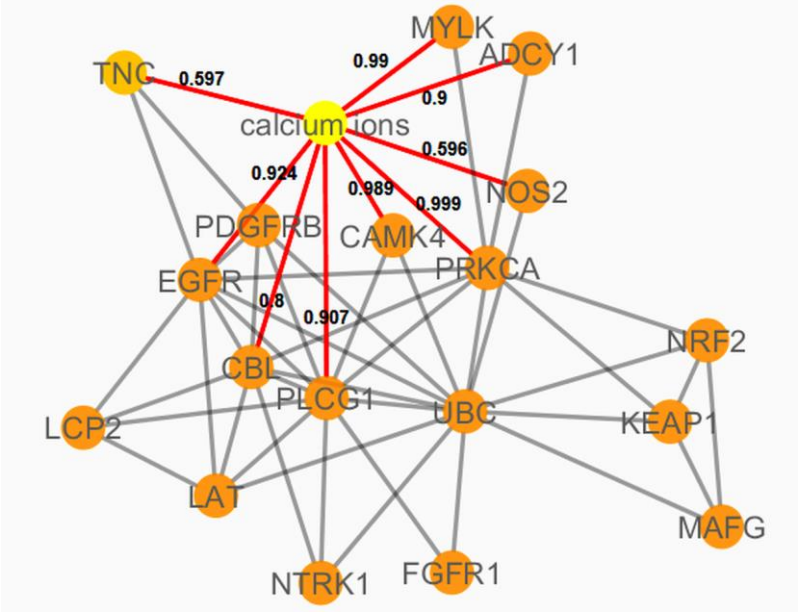


Fig. 1 Calcium ions bind to several proteins indicated by red line, numbers associate with probable or power of connection

This interaction network data was validated based binding score range from 0 until 1. The higher score means more reliable. The binding score was evaluated from textmining, experiments, databases, co-expression, neighborhood, gene fusion, co-occurrence, and predictions. The direct interaction was considered if the binding score above 0.8. This computational study considers 80% accuracy of interaction prediction. 8 protein that

predicted have direct interaction will further analyzed based on the correlation with NRF2. Classical PKCs (a, bI, bII and c) are activated, at least, at three different sites; by diacylglycerols interacting with the C1 domain, by Ca^{2+} acting at the C2 domain, which bridges with membrane phospholipids, especially phosphatidylserine and by PIP2, which also acts at the C2 domain [6]. Ca^{2+} binding allosterically destabilizes the terminal regions of C2a and thereby facilitates the conformational rearrangement necessary for full membrane insertion and activation of PRKCA [17].

NRF2 activation initiated by Ca^{2+} binds to PRKCA

NRF2 activation is the main process to counter the oxidative stress. The pathway was generated to know the protein that has directly interaction with NRF2. The bridge between Ca^{2+} to activate the NRF2 was mediated by PRKCA (Fig. 2).

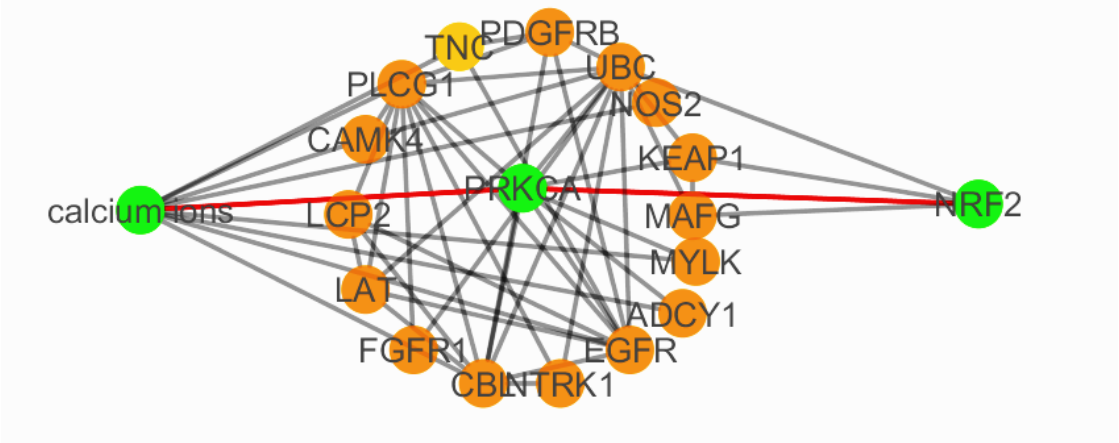


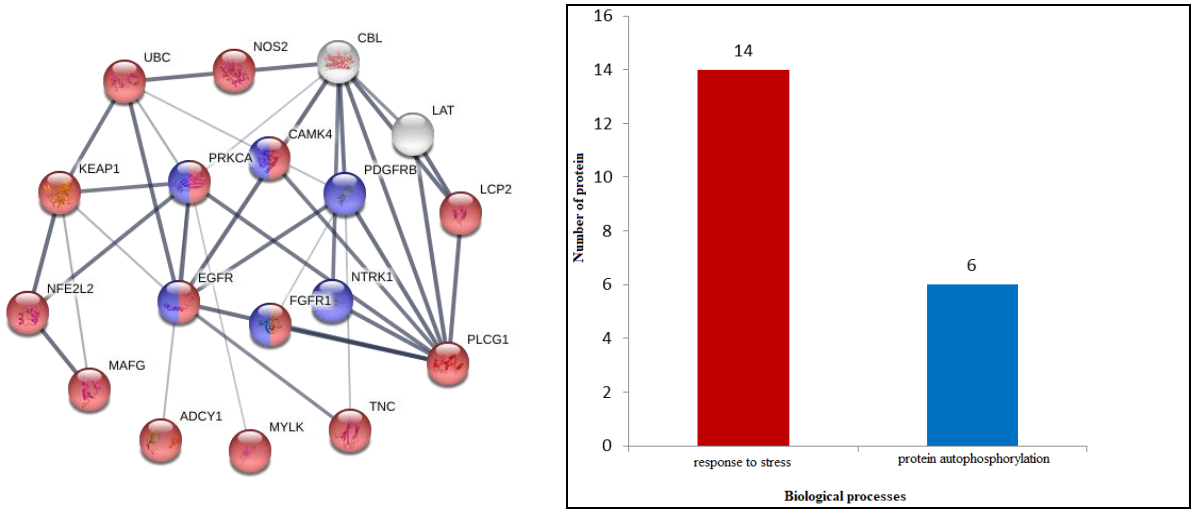
Fig. 2 Connection between Ca^{2+}, PRKCA and NRF2 (red line)

Activation prediction at Fig. 2 above is 0.9. It means there is strong connection between PRKCA and NRF2. NRF2, which is the representative antioxidant and cytoprotective factor involved in cancer chemoprevention. PKC pathways are particularly involved in the activation of NRF2 by GKB (gymnasterkoreayne B), followed by translocation of NRF2 and induction of NQO-1 (NAD(P)H dehydrogenase quinone 1). These results suggested that GKB induces NRF2 translocation and expression by differential regulation of ERK and PKC pathways in HCT116 cells. PKC regulates stability and translocation of NRF2 by phosphorylation of Ser-40 (α-amino acid serine 40). Since phosphorylation of NRF2 might protect from interacting with Keap1 (Kelch-like ECH-associated protein), results in the activation and expression of NRF2. The increasing of NRF2 released from Keap1 promoted translocation of NRF2 to the activation of phase II detoxification enzymes [15]. Phosphorylation of wild-type NRF2 by PKC promoted its dissociation from Keap1, whereas the NRF2-S40A mutant remained associated. These findings suggest that the PKC-catalyzed phosphorylation of NRF2 at Ser-40 is a critical signaling event leading to ARE-mediated cellular antioxidant response [10]. Phosphorylation of NRF2 Ser-40 by aPKC(s) is involved in the nuclear translocation and ARE transactivation of NRF2 by oxidative stress [19]. Protein kinase C has been shown to phosphorylate NRF2 in its Neh2 (NRF2-ECH homology 2) domain at Ser-40, disrupting the association between NRF2and Keap1 thus promoting the translocation of NRF2 into the nucleus [2].

The functional network was proteins data that previously analyzed with Ca^{2+} interaction. Biological processes clearly revealed that all proteins take a role in two mechanism that are: (1) Response to stress and (2) protein autophosphorylation (Fig. 3). It assumes that higher level of Ca^{2+} in cytoplasmic could activate protein that responsible for facing the stress condition.

The result showed that there are 14 proteins labelled red on the network could act to response the stress. In addition, there are 6 proteins that have a function as protein autophosphorylation. This type of action is important to activate some proteins by phosphorylation process.

This study confidently stated that PKC take a crucial part in the activation of NRF2. PKC was found in two types of biological processes that evaluated in this study. PKC is a family of serine/threonine kinases which can be subdivided into 3 classes; classical, novel and atypical. Atypical isoform is responsible for the phosphorylation and nuclear translocation of NRF2 to induce phase II cytoprotective proteins [2]. Micro and macrovascular complications in diabetic patients result from chronic activation of protein kinase C (PKC), the enzyme involved in controlling other protein functions. PKC has been associated with vascular changes such as increased permeability, contractility, extracellular matrix synthesis, cell growth and apoptosis, angiogenesis, leukocyte adhesion, cytokine activation and inhibition. Disturbance of vascular cell homeostasis caused by different PKC isoforms (PKC-α, -β1/2, and PKC-δ) associated with complications large vessels (atherosclerosis, cardiomyopathy) and small vessels (retinopathy, nephropathy and neuropathy [21].



A. Functional pathway B. Biological processes of pathway

Fig. 3 Functional and biological analysis effect Ca^{2+} to PRKCA and NRF2.
Red color as response proteins to stress condition and blue color as proteins autophosporilation

Major isoforms that underwent changes in the diabetic condition are PKCa in the nerve and PKCb in the epineurial artery [29]. Baicalein, one of the major flavonoids, prevented PC12 cells from 6-OHDA-induced oxidative damage via the activation of Keap1/NRF2/HO-1, and it also involves the PKCα and PI3K/AKT signaling pathway [30]. NRF2 knockout (NRF22/2) results in impaired capacity to replenish glutathione stores, compounded with a decreased detoxification capability, highlights the importance of NRF2 in the regulation of glutathione synthesis and cellular detoxification processes [4]. Beside NRF2 role against oxidative stress and have cytoprotective effect, NRF2 molecule can also independently controls the expression of genes responsible for many aspects of cellular metabolism in diabetic mellitus [5].

Conclusion

In diabetic neuropathy, chronic hyperglycemia will produce intracellular Ca^{2+} disorders, Ca^{2+} ions can bind to some proteins, one of them is PRKCA as the most powerful protein bonding. As known as NRF2 neuroprotective in diabetic neuropathy also increased. Whether Ca^{2+} is associated with NRF2 in diabetic neuropathy, from data above, the researchers concluded that activation NRF2 by Ca^{2+} intracellular which increases in diabetic condition through PRKCA, PRKCA phosphorylate NRF2 in its Neh2 domain at Ser-40, followed by dissociation NRF2 from Keap1 then promoting the translocation of NRF2 into the nucleus. NRF2 activates a myriad of genes that protect cells against oxidative stress caused by diabetic condition. From functional and biological analysis, the effect of Ca^{2+} through PRKCA and NRF2, PRKCA and NRF2 have role in response of stress condition and autophosphorylation.

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