2012 International Conference on Clean and Green Energy
IPCBEE vol.27 (2012) © (2012) IACSIT Press, Singapore

Analysis of the Relationship between Human SIRT4 and Glutamate Dehydrogenase Using a Yeast Model System

Lan Zhao [1] and Pil jae Maeng [1 +]
[1] Molecular Cell Biology Lab. Chungnam National University

Abstract. SIRT4 is a mitochondrial ADP-ribosyltransferase that inhibits mitochondrial glutamate dehydrogenase 1 activity in beta cell mitochondria. The alteration of the activity of GDH in yeast could influence the ability of resistance of yeast to ROS. So first we insert SIRT4 into series of GDHs deletion yeast strains, to confirm whether SIRT4 could affect the survival ratio of the yeast under the oxidative stress. Then build SIRT4,hGDH model in GDH deleted yeast strain. By utilizing such models to investigate the relationship between SIRT4, hGDH and ROS.

Keywords: yeast, GDH, SIRT4, ROS.

1. Introduction

Diabetes mellitus, often simply referred to as diabetes, are a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

There are mainly 2 types of diabetes:

Type 1 diabetes: results from the body's failure to produce insulin, is an absolute lack of insulin, whereas type 2 diabetes: results from insulin resistance, in the type2 diabetes, insulin expression normally, some patients even showed a higher level of insulin. And type 2 diabetes is characterized by the ectopic ROS expression and hyperinsulinism. Insulin resistance may happen before diabetes, induce the compencatory increase of insulin to keep the level of blood sugar. But when the sensibility of body to insulin keep reducing, the body can not response to the high level of blood sugar any more, even the insulin expresses normally. And hyperglycemia and high FFA (free fatty acid) could induce a mass of ROS. On one side, ROS aggravates insulin resistance, on the other side, ROS decrease insulin gene expression and secretion by damage the β-cell and finally bring about apoptosis[1,2]. Hyperinsulinism is induced by insulin resistance and it is suggested that hyperinsulinism contributes to the hypertension.

Sirtuin 4, also known as SIRT4 is a protein which in humans is encoded by the SIRT4 gene. This gene encodes a member of the sirtuin family of proteins which are homologs of the Sir2 gene in budding yeast. Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes. The functions of human sirtuins have not yet been fully determined; however, yeast sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA. Studies suggest that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. The protein encoded by this gene is included in class IV of the sirtuin family[3].

SIRT4 is a mitochondrial ADP-ribosyltransferase that inhibits mitochondrial glutamate dehydrogenase 1 activity, thereby down-regulating insulin secretion in response to amino acids. It has been shown that SIRT4 regulates fatty acid oxidation and mitochondrial gene expression in liver and muscle cells.

[+] Corresponding author. Tel.: + 042-821-6415; fax: +82-042-822-7367.
E-mail address: pjmaeng@cnu.ac.kr.

Glutamate dehydrogenase is an enzyme, present in most microbes and the mitochondria of eukaryotes, as are some of the other enzymes required for urea synthesis. GDH of yeast, there are 3 types of GDH gene in yeast, GDH1,2 and 3. GDH1,3 are in charge of the generation of glutamate from alpha-KG, GDH2 regulates a opposite reaction.

Alpha-KG participates in the TCA cycle, which is responsible for the generation of ATP. Some researches have proved that alpha-KG is related to the ROS generation. In the TCA cycle, alpha-KG forms alpha-KGDH, which is the target and generator of ROS. Tretter L[8] proved that alpha-KGDH is able to generate ROS, and this reaction is regulated by the NADH/NAD^+ ratio(Fig. 1).

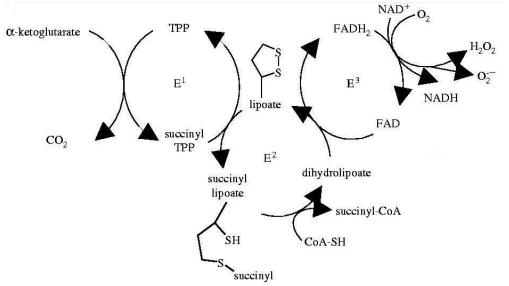

Fig. 1: Generation of reactive oxygen species by α-KGDH

GSH, glutathione, is a tripeptide that contains an unusual peptide linkage between the amine group of cysteine, a downstream product of glutamate. Armstrong demonstrated that decrease in GSH could activate the apoptosis signaling, and increased ROS production following a GSH depletion, which indicates that the GSH plays an important role in the ROS elimination(Fig. 2)[4].

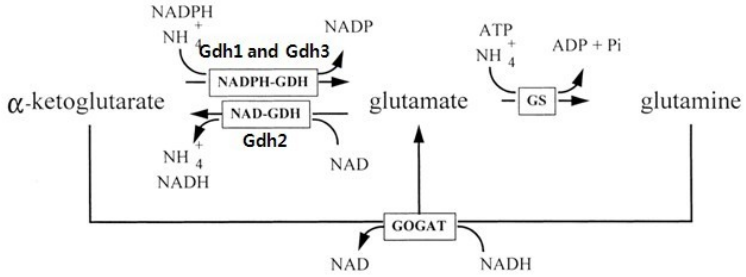

Fig. 2: illustration of glutamine and glutamate metabolism

Glutamate dehydrogenase (GDH) is a mitochondrial enzyme that catalyzes the oxidative deamination of glutamate to alpha-ketoglutarate using NAD or NADP as cofactors[5]. Oxidative deamination of glutamate by GDH supplies alpha-KG to the TCA cycle and generates ATP. This increases in the ATP/ADP ratio triggers the closure (inhibition) of the potassium channel (K_{ATP} channel), and then induce the plasma membrane depolarization. This depolarization opens voltage-gated Ca^{2+} channel. Ca^{2+} influx, a rise in intracellular calcium concentration ($[Ca^{2+}]i$), and thereby the resultant elevation of $[Ca^{2+}]i$ signals the fusion of secretion vesicles, loaded with insulin, triggers exocytosis and insulin release from the insulin containing granules located in pancreatic beta cells.

Marcia C. Haigis proved that SIRT4 uses NAD to ADP-ribosylate and down-regulate glutamate dehydrogenase (GDH) activity in β cell mitochondria[6]. Nidhi Ahuja proved that SIRT4 could down regulate the secretion of insulin[7]. And we found when GDH2 was inhibited in yeast, the survival ratio increased compared to the wild type under the presence of ROS. GDH3 deleted strain showed a lower survival ratio than wild type under the ROS stress, and adding GSH, a downstream product of glutamate, could rescue the death of cells caused by oxidative damage.

So we speculate that, in the type 2 diabetes, on one side, SIRT4 could down regulate the expression of insulin by reducing the alpha-KG generation, which participates in the TCA cycle, helps the expression of ATP. And simultaneously, SIRT4 helps the cells to fight against the oxidative damage. These progresses are

base on the inhibition of SIRT4 to hGDH. Once hGDH activity is inhibited by SIRT4, alpha-KG generation is reduced, and glutamate consumption decrease, this indicates that insulin expression will be limited and GSH level could be kept at a relative stable level, then GSH will participate in the elimination of ROS. And on the other side, down expression of alpha-KG led to the reduce of alpha-KGDH, and finally caused the decrease of ROS generation too. The down regulation of insulin expression and elimination of ROS, these two results could relieve the symptom of type 2 diabetes. To prove this, we use ROS and ectopic GDH expression to build a type 2 diabetes model in yeast, then, utilize this model to investigate the function and relationship between SIRT4, ROS and human GDH.

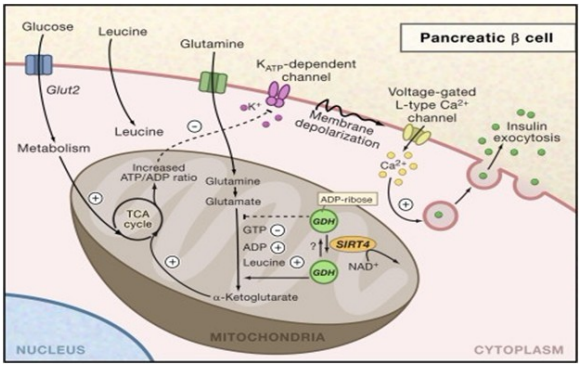

Fig. 3: insulin expression regulated by Sirt4

2. References

[1] Weir GC, Laybutt DR, Kaneto H, Bonner-Weir S, Sharma A. 2001. β cell adaptation and decompensation during the progression of diabetes. *Diabetes* (supplement 1): S154–S159.

[2] Prentki M, Nolan CJ. 2006. Islet a cell failure in type 2 diabetes. *The Journal of Clinical Investigation.* 116(7): 1802–1812.

[3] Tanny JC, Dowd GJ, Huang J, Hilz H, Moazed D. 1999. An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. *Cell*. 99 (7): 735–45.

[4] Armstrong JS, Steinauer KK, Hornung B, Irish JM, Lecane P, Birrell GW, Peehl DM, Knox SJ. Role of glutathione depletion and reactive oxygen species generation in apoptotic signaling in a human B lymphoma cell line. *Cell Death Differ*. 2002 Mar;9(3):252-63.

[5] Smith EL, Austin BM, Blumenthal KM, Nyc JF. 1975. Glutamate dehydrogenase. In: Boyer PD, editor. *The enzymes New York: Academic Press*. 11: 293–367.

[6] Marcia C. Haigis, Raul Mostoslavsky, Kevin M. Haigis, Kamau Fahie, p Danos C. Christodoulou, Andrew J. Murphy, David M. Valenzuela, George D. Yancopoulos, Margaret Karow, Gil Blander. SIRT4 Inhibits Glutamate Dehydrogenase and Opposes the Effects of Calorie Restriction in Pancreatic â Cells. *Cell.* 126(5): 941 - 954.2006.

[7] J Nidhi Ahuja, Bjoern Schwer, Stefania Carobbio, David Waltregny, Brian J. North, Vincenzo Castronovo, Pierre Maechler and Eric Verdin. Regulation of Insulin Secretion by SIRT4, a Mitochondrial ADP-ribosyltransferase. *The Journal of Biological Chemistry*. 2007, 282, 33583-33592.

[8] Tretter L, Adam-Vizi V. Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress.*Philos Trans R Soc Lond B Biol Sci*.2005, 360(1464):2335-45.