

Spermidine may decrease ER stress in pancreatic beta cells and may reduce apoptosis via activating AMPK dependent autophagy pathway

P.B. Tirupathi Pichiah^c, U. Suriyakalaa^a, S. Kamalakkannan^b, P. Kokilavani^a, S. Kalaiselvi^a, D. SankarGanesh^a, J. Gowri^d, G. Archunan^b, Youn-Soo Cha^c, S. Achiraman^{a,*}

^aDepartment of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India

^bDepartment of Animal Science, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India

^cDepartment of Food Science and Human Nutrition, Chonbuk National University, Jeonju, South Korea

^dDepartment of Biochemistry, Seethalakshmi Ramaswami College, Tiruchirappalli, Tamil Nadu, India

ARTICLE INFO

Article history:

Received 11 December 2010

Accepted 4 July 2011

ABSTRACT

The risk for diabetes increases with increasing BMI < 25. Insulin resistance is the key factor for type 2 diabetes; studies revealed that endoplasmic reticulum stress is the main factor behind this disease. With increase in ER stress, pancreatic beta cells start to undergo apoptosis, leading to a decline in the pancreatic beta cell population. The ER stress arises due to unfolded protein response. Recently, spermidine get importance for increasing the longevity in most of the eukaryotes including yeast, *Caenorhabditis elegans*, *Drosophila* and human peripheral blood mononuclear cells via induction of autophagy pathway. Autophagy is also involved in regulation of scavenging of proteins. One of the major cellular pathways for scavenging the aggregated intracellular protein is autophagy. Hence spermidine can be a candidate for the treatment type 2 diabetes. Autophagy genes are regulated by mTOR (mammalian Target Of Rapamycin) dependent or independent pathway via AMPK. Hence either inhibition of mTOR or activation of AMPK by spermidine will play two crucial roles, first being the activation of autophagy and secondly the reduction of endoplasmic reticulum stress which will reduce beta cell death by apoptosis and thus can be a novel therapeutic candidate in the treatment of insulin resistance in type 2 diabetes and preserving pancreatic beta cell mass.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

Diabetes is spreading worldwide as an epidemic. Insulin resistance is a key factor behind type 2 diabetes. The pancreatic beta-cell senses nutrients, neurotransmitters and hormones in the circulating blood. The unique function of the cell is to integrate all these ambient signals into an appropriate insulin secretory rate in order to maintain normal glucose homeostasis [1]. Insulin resistance and decreased insulin production by pancreatic beta-cells are recognized as the primary defects in type 2 diabetes [2]. In many pre-diabetic insulin-resistant individuals, the beta-cells often produce excessive quantities of insulin in order to compensate for insulin resistance. Evidences suggest that endoplasmic reticulum stress is the cause behind insulin resistance state [3,4]. Endoplasmic reticulum (ER) stress occurs when protein folding and modification are disrupted; triggering a complex protective response termed the unfolded protein response (UPR) [5]. The unfolded protein response (UPR) play a dual role in beta-cells, acting as benefi-

cial regulators under physiological conditions or as triggers of beta-cell dysfunction and apoptosis under situations of chronic stress [6]. Novel findings suggest that chronic high glucose and fatty acid exposure, contribute to beta-cell failure in type 2 diabetes. Endoplasmic reticulum (ER) stress is necessary for the promotion of apoptosis in beta cells in both animal models of diabetes and of humans with type 2 diabetes [7]. When unfolded protein accumulates secondary to a mild stress, universal protein synthesis is inhibited, but then resumes upon recovery, but in more severe or persistent stress would lead to apoptosis [8]. Reducing the rate of synthesis of protein will be helpful in restoring the endoplasmic reticulum stress, and scavenging of unfolded proteins through autophagy will be a benefit in recovery of the cell from the stressful condition. The regulation of protein synthesis and autophagy comes under the governance of mTOR. The Eukaryotic Initiation Factor 4 (eIF4) groups are the downstream target for mTOR, these factors mediate key steps in translation initiation, such as the recruitment of the mRNA to the small (40S) ribosome subunit and the recruitment of the initiator methionyl-tRNA (Met-tRNAs) that recognizes the start codon at the beginning of the coding region [9].

Spermidine is a polyamine compound, found in ribosomes and living tissues, originally it was isolated from semen from which

* Corresponding author. Address: Department of Environmental Biotechnology, School of Environmental Science, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India. Tel.: +91 431 2407088; fax: +91 431 2407045.

E-mail address: achiramans@gmail.com (S. Achiraman).

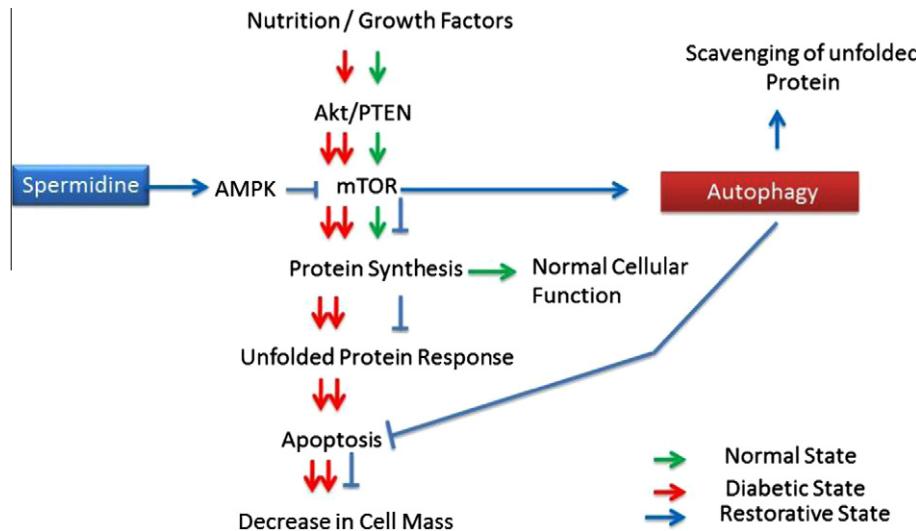


Fig. 1. The role of spermidine in controlling apoptosis. Spermidine, can activate autophagy by interacting with mTOR via AMPK. Activated autophagy will scavenge the unfolded proteins, that can help in restoration of unfolded protein response that can help to overcome and prevent apoptosis in beta pancreatic cell.

it derived its name. Spermidine is a triamine structure that is produced by spermidine synthase (SpdS) which catalyzes mono-alkylation of putrescine (1,4-diaminobutane) with decarboxylated S-adenosylmethionine (dcAdoMet) 3-aminopropyl donor [10]. The formal alkylation of both amino groups of putrescine with the 3-aminopropyl donor yields the symmetrical tetraamine spermine [11].

Decrease in polyamine level is found to induce pancreatitis [12], polyamine supplementation is found to reduce chemical induced pancreatic inflammation [13] indicating that the polyamine level should be in optimal level, and decrease of this level may lead to disorders. Pancreatic spermidine level in pancreas of diabetic animals were found to be lower comparing to the normal animals [14].

Autophagy is a mechanism in which portions of the cytoplasm are sequestered within autophagosomes and then targeted to lysosomes for digestion. Recent evidence suggests that autophagy usually mediates cytoprotection, thereby avoiding the apoptotic or necrotic demise of stressed cells [15,16].

Spermidine is reported to increase the life span in many model organism including nematodes (*Caenorhabditis elegans*), yeast (*Saccharomyces cerevisiae*), and flies (*Drosophila melanogaster*) and significantly reduces age related oxidative protein damage in mice [17].

A recent study revealed that, administration of spermidine, a natural polyamine whose intracellular concentration declines during human ageing, markedly extended the lifespan of yeast, flies and worms, and human immune cells through induction of autophagy [18]. Study led by Morselli et al. showed that spermidine do not affect the phosphorylation of mTOR and neither effects its substrate ribosomal protein S6 kinase, which reveals that spermidine induces autophagy through AMPK dependent pathway [19].

AMPK/mTOR in protein synthesis and autophagy regulation

AMP-activated protein kinase (AMPK) inhibits mTOR in response to reduced ATP levels [20]. AMP activated protein kinase suppress protein synthesis by down regulating the mTOR signaling cascade [21–23]. mTOR regulates numerous components involved in protein synthesis, including initiation and elongation factors,

and the biogenesis of ribosomes themselves [24]. Low insulin/IGF-1 signaling, nutrient or energy deprivation, and stress converge to down regulate the activity of the protein kinase target of rapamycin (TOR) [25]. Inhibition of mTOR stops the protein synthesis machinery due to inactivation of its effector, p70 S6 kinase and activation of the eukaryotic initiation factor 4E binding protein 1 (4E-EP1), an inhibitor of translation [26]. Autophagy associated genes are under the regulation of mTOR [27]. Rapamycin which is an inhibitor of TOR, enhances autophagy by inhibition of the TOR [28]. Suppressing mTOR activity induces autophagy, a lysosomal catabolic pathway for turnover of proteins and organelles.

Activation of autophagy inhibits apoptosis- and vice versa

There are enough publications as an evidence to support the fact that activation of autophagy reduces or inhibit the apoptosis and vice versa. AMPK is an energy sensor that mediates autophagy and inhibits apoptosis [29]. Whereas; autophagy inhibition increases the chances of apoptosis. The genetic inhibition of autophagy by knockout or knockdown of ATG genes often lead to apoptotic or necrotic death of cells [30–32], chemical inhibition of autophagy through PI3K/Akt using sodium selenite increases NB4 cell apoptosis [33], which clearly reveals that inhibition of autophagy contributes to the up-regulation of apoptosis. Autophagy inhibition enhances anthocyanin-induced apoptosis in hepatocellular carcinoma [34]. Thus, activation of autophagy inhibits apoptosis and inhibition of autophagy activates apoptosis.

Hypothesis

Spermidine had shown to induce autophagy in most eukaryotes. Hence spermidine can be used to induce autophagy in pancreatic beta cells (Fig. 1), which can reduce the stress load raised due to unfolded protein response. Autophagy induction is an indirect message that protein synthesis is in suspended state, hence it is possible it could benefit in restoration of endoplasmic reticulum stress raised due to unfolded protein response. This will provide sufficient time for the beta cells to recover and the apoptosis induced beta cell death will be reduced. Hence spermidine can be a potent candidate in preserving beta cell mass which are reduced due to apoptosis induced cell death in type 2 diabetes mellitus.

Conflict of interest

None declared.

References

- [1] Henrik Ortsäter A, Åke Sjöholm. A busy cell – endoplasmic reticulum stress in the pancreatic β -cell. *Mol Cell Endocrinol* 2007;277:1–5.
- [2] Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988;37:1595–607.
- [3] Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004;306:457–61.
- [4] Nakatani Yoshihisa, Kaneto Hideaki, Kawamori Dan, Yoshiuchi Kazutomi, Hatazaki Masahiro, Matsuoka Taka-aki, et al. Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. *J Biol Chem* 2005;280: 847–51.
- [5] Schröder M, Kaufman RJ, et al. The mammalian unfolded protein response. *Ann Rev Biochem* 2005;74:739–89.
- [6] Eizirik Décio L, Cardozo Alessandra K, Cnop Miriam. The Role for Endoplasmic Reticulum Stress in Diabetes Mellitus. *Endocrine Rev* 2008;29:42–61.
- [7] Laybutt DR, Preston AM, Akerfeldt MC, Kench JG, Busch AK, Biankin AV, et al. Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia* 2007;50(4):752–63.
- [8] Knowlton AA. Life, death, the unfolded protein response and apoptosis. *Cardiovas Res* 2007;73(1):1–2.
- [9] Gingras AC, Raught B, Sonenberg N. EIF4 translation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem* 1999;68:913–63.
- [10] http://www.biosynth.com/index.asp?topic_id=162&g=19&m=205.
- [11] Jänele J, Alhonen L, Pietilä M, Keinänen TA. Genetic approaches to the cellular functions of polyamines in mammals. *Eur J Biochem* 2004;271:877–94.
- [12] Hyvönen MT, Keinänen TA, Cerrada-Gimenez M, Sinervoirta R, Grigorenko N, Khomutov AR, et al. Role of hypusinated eukaryotic translation initiation factor 5A in polyamine depletion-induced cytostasis. *J Biol Chem* 2007;282(48): 34700–6.
- [13] Jin HT, Lämsä T, Hyvönen MT, Sand J, Räty S, Grigorenko N, et al. A polyamine analog bis(methylspermine ameliorates severe pancreatitis induced by intraductal infusion of taurodeoxycholate. *Surgery* 2008;144(1):49–56.
- [14] Méndez JD, Arreola MA. Effect of L-arginine on pancreatic arginase activity and polyamines in alloxan treated rats. *Biochem Int* 1992;28(4):569–75.
- [15] Madeo F, Tavernarakis N, Kroemer G. Can autophagy promote longevity? *Nat Cell Biol* 2010;12(9):842–6.
- [16] Morselli E, Galluzzi L, Kepp O, Criollo A, Maiuri MC, Tavernarakis N, et al. Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol. *Aging (Albany NY)* 2009;1(12):961–70.
- [17] Madeo F, Eisenberg T, Büttner S, Ruckenstein C, Kroemer G. Spermidine: a novel autophagy inducer and longevity elixir. *Autophagy* 2010;6(1):160–2.
- [18] Eisenberg T, Knauer H, Schauer A, Büttner S, Ruckenstein C, Carmona-Gutierrez D, et al. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* 2009;11(11):1305–14.
- [19] Morselli E, Mariño G, Bennetzen MV, Eisenberg T, Megalou E, Schroeder S, et al. Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. *J Cell Biol* 2011;192(4):615–29.
- [20] Poels J, Spasic MR, Callaerts P, Norga KK. Expanding roles for AMP-activated protein kinase in neuronal survival and autophagy. *Bioessays* 2009;31:944–52.
- [21] Bolster DR, Crozier SJ, Kimball SR, Jefferson LS. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J Biol Chem* 2002;277: 23977–80.
- [22] Ning Junyu, Clemons David R. AMP-activated protein kinase inhibits IGF-I signaling and protein synthesis in vascular smooth muscle cells via stimulation of insulin receptor substrate 1 S794 and tuberous sclerosis phosphorylation. *Mol Endocrinol* 2010;6:1218–29.
- [23] Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003;115:577–90.
- [24] Wang Xuemin, Proud CG. The mTOR pathway in the control of protein synthesis. *Physiology* 2006;21:362–9.
- [25] Wullschleger S, Loewenthal R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006;124:471–84.
- [26] Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* 2002;10(1): 151–62.
- [27] Rosenbluth JM, Pietenpol JM. mTOR regulates autophagy-associated genes downstream of p53 2009;5:114–6.
- [28] Floto RA, Sarkar S, Perlstein EO, Kampmann B, Schreiber SL, Rubinstein DC. Small molecule enhancers of rapamycin-induced TOR inhibition promote autophagy, reduce toxicity in Huntington's disease models and enhance killing of mycobacteria by macrophages. *Autophagy* 2007;3(6):620–2.
- [29] Harhaji-Trajkovic L, Vilimovich U, Kravcic-Stevovic T, Bumbasirevic V, Trajkovic V. AMPK-mediated autophagy inhibits apoptosis in cisplatin-treated tumor cells. *J Cell Mol Med* 2009;13:3644–54.
- [30] Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, Thompson CB. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. *Cell* 2005;120:237–48.
- [31] Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* 2008;9:1004–10.
- [32] Boya P, Gonzalez polo RA, Casares N, Perfettini JL, Dessen P, Larochette N, et al. Inhibition of macroautophagy triggers apoptosis. *Mol Cell Biol* 2005;25:1025–40.
- [33] Ren Yuv, Huang Fang, Liu Yuan, Yang Yang, Jiang Qian, Xu Caimin. Autophagy inhibition through PI3K/Akt increases apoptosis by sodium selenite in NB4 cells. *BMB Rep* 2009;42(9):599–604.
- [34] Longo Luigia, Platini Francesca, Scardino Anna, Alabiso Oscar, Vasapollo Giuseppe, Tessitore Luciana. Autophagy inhibition enhances anthocyanin-induced apoptosis in hepatocellular carcinoma. *Mol Cancer Ther* 2008;7(8): 2476–85.