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SIRT1: Importance of *O*-GlcNAc and Phosphorylation Interplay in Aging and Diabetes

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Abstract.- Sirtuins are conserved protein deacetylases or ADP ribosyltransferases acting on broad range of cellular proteins. The target proteins are present in the nucleus, cytoplasm, and mitochondria and are post-translationally modified by acetylation via Silent Mating Type Information Regulation 2 Homolog 1 (SIRT1). Among the rest of the sirtuins, SIRT1 is most studied and is involved in the process of aging and controls the extent of life span. Different post-translational modifications (PTMs) such as phosphorylation and glycosylation control the function of SIRT1. In the present study, the role of these modifications and their interplay, involving the regulation of the transcriptional activity of SIRT1, has been examined *in silico*. The current study suggests that transcriptional activity of SIRT1 is increased by phosphorylation, while glycosylation decrease it. During aging and diabetes, addition of *O*-GlcNAc attenuates the activity of SIRT1 on the function of related proteins. These PTMs play an important role in regulation of various activities of SIRT1 which range from homeostasis, survival and aging.

Keywords: SIRT1, YinYang, phosphorylation, *O*-GlcNAc, PTM, aging, anti-aging, life span.

INTRODUCTION

Sirtuins are a set of highly conserved proteins, present in many organisms, ranging from archaea to mammals (Westphal *et al.*, 2007; Blander and Guarente, 2004). They are class III histone/protein deacetylases (HDAC), which require coenzyme NAD+ for activation (Gray and Ekstrom, 2001; Imai *et al.*, 2000; Tanner *et al.*, 2000). In mammals, they make up a family of seven members known as SIRT1 to SIRT7 (Haigis and Guarente, 2006). SIRT1 and its family members promote longevity in different organisms, such as yeast, worms, flies and mice (Longo and Kennedy, 2006; Leibiger and Berggren, 2006; Lin and Guarente, 2003).

Silent Mating Type Information Regulation 2 Homolog 1 (SIRT1) is the most studied member of the sirtuin family. It deacetylates different transcription factors, and removes acetyl groups from several histone and non-histone proteins and therefore influences a wide range of physiological functions (Michan and Sinclair, 2007; Haigis and Guarante, 2006; Yamamoto *et al.*, 2007; Pillarisetti, 2008). SIRT1 is a major regulator of gene expression and SIRT1-mediated histone deacetylation is associated with the formation of heterochromatin while SIRT1-mediated non-histone proteins function as transcriptional activators, or transcriptional repressors or regulators thereof (Levine *et al.*, 2006; Anastasiou and Krek, 2006).

SIRT1 catalyzes an enzymatic reaction that generates nicotinamide and the acetyl group of the substrate is transferred to cleaved NAD, generating a unique metabolite, *O*-acetyl-ADP ribose (Pillarisetti, 2008). Changes in cellular NAD levels, or the NAD-NADH ratio, are the primary mechanisms regulating SIRT1 activity (Guarente and Picard, 2005). The list of SIRT1 substrates is continuously increasing and includes numerous transcription factors for example: p53, members of the FOXO family, hairy and enhancer of split 1 (HES 1), hairy/enhancer-of-split related with YRPW motif 2 (HEY2) , peroxisome proliferator - activated receptor gamma (PPAR γ), chicken ovalbumin upstream promoter transcription factor (COUPTF)-interacting protein 2 (CTIP 2), p300, PGC-1 α (PPAR γ coactivator), and nuclear factor κB (NFκB) (Michan and Sinclair, 2007; Pillarisetti, 2008;

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Haigis and Guarente, 2006; Yamamoto *et al.*, 2007).

Aging is defined as the gradual biological impairment of normal functions resulting from cellular changes and structural modifications caused by environmental changes accumulating over a given time period (Hipkiss, 2006b). These changes have a direct impact on the functional ability of organs, biological systems and ultimately the organism as a whole (Hipkiss, 2006a; Schoneich, 2006). These changes are complex biochemical processes that also affect protein modifications, such as phosphorylation and glycosylation (Marton *et al.*, 2011). SIRT1 plays an important role in several age-relayed diseases like neurodegenerative diseases (Luchsinger, 2006). SIRT1 protects axons from damage in animal models of the Parkinson's disease (Wallerian degenerative disease) (Wu *et al.*, 2011). Furthermore, the use of resveratrol (a SIRT1 activator) in models of Huntington's disease shows that SIRT1 is able to reduce cell death by inhibition of NF-κB signalling (Chen *et al.*, 2005). Alzheimer's disease has also been linked to SIRT1 function and calorie restriction in monkeys (Qin *et al.*, 2006)

Post-translational modifications (PTMs) are critical functional regulators in biological systems. The interplay of PTMs in regulation of transcriptional activities of various factors has been described (Kaleem *et al.*, 2008, 2009, 2011; Nasir-ud-Din *et al.*, 2010). An important modification is the *O*-GlcNAc modification that functionally opposes phosphorylation (Walsh, 2006). *O*-GlcNAc modification occurs on the hydroxyl function of Ser/Thr. A complex relation between phosphorylation and *O*-GlcNAc modification on same or neighboring residues are called Yin Yang sites. These alternative modifications of Ser/Thr residues often result in changing the function of a protein. Sometimes, Ser/Thr residues show a very high potential for either *O*-GlcNAc modification or phosphorylation, or show a potential very close to the specific threshold value predicted by the existing methods. When a residue is conserved and shows a very high potential for phosphorylation as well for *O*-GlcNAc modification very close to the threshold value, it is considered as a false negative Yin Yang site, as O-GlcNAc transferases (OGT) and kinases may have an equivalent accessibility to such a residue to modify it (Kaleem *et al.*, 2011)

In the current investigation, interplay of PTMs between phosphorylation and *O*-GlcNAc modification of the mammalian SIRT1 has been investigated using bioinformatic tools. This *in silico* study suggests that phosphorylation accelerates the anti-aging process of specific proteins, whereas O-GlcNAc does the opposite.

MATERIALS AND METHODS

The sequence of SIRT1 of *Mus musculus* was retrieved from Swiss-Prot (Boeckmann *et al.*, 2003) with an accession number Q923E4. BLAST search was performed using NCBI database of non-redundant sequences using all default parameters (Altschul *et al.*, 1997).The search resulted in 9 selected orthologs of different animals: mammals (*Mus musculus* Q923E4, *Homo sapiens* NP_036370.2, *Bos taurus* NP_001179909.1, Sus *scrofa* NP_001139222.1), birds *(Gallus gallus* NP_001004767.1), amphibians *(Xenopus laevis* NP_001091195.1); *Xenopus silurana tropicalis* CAL49361.1), fishes (*Notobranchius furzeri* ABX71822.1, *Notobranchius kuhntae* ACB30548.1).

The chosen sequences were multiply aligned using ClustalW2 with all default parameters (Thompson *et al.*, 1994) (http://www.ebi.ac.uk/clustalw/). The glycosylation and Yin Yang sites of SIRT1 in *Mus musculus* were predicted using YingOYang 1.2 (Gupta and Brunak, 2002) (http://www.cbs.dtu.dk/services/YinOYang/). The phosphorylation sites were determined using NetPhos 2.0 (Blom *et al.*, 1999) (http://www.cbs.dtu.dk/services/NetPhos 2.0/)

The predictions of PTM using different computational models are useful for the interpretation of the functional regulation controlled by PTMs. In this study different computational prediction models utilized for the prediction of SIRT1 in *Mus musculus*. These computational models based on different non-homology methods such as NetPhos 2.0 was developed by training the neural networks with phosphorylation data from Phosphobase 2.0. A threshold value of 0.5 is used by Netphos 2.0 to suggest the possibility of

phosphorylation. YinOYang 1.2 employs a sequence data to train a jury of neural networks on 40 experimentally determined *O*-GlcNAc acceptor sites to define the sequence environment and surface accessibility. Moreover, this method has the ability to predict the sites known as Yin Yang sites that may be *O*-GlcNAc modified and phosphorylated alternatively. The threshold value used by YinOYang 1.2 varies depending upon surface accessibility of the different amino acid residues. False negative sites are identified as well, by coupling conservation importance and modification potential of the two methods.

RESULTS AND DISCUSSION

The interplay between PTMs such as phosphorylation, glycosylation, acetylation and methylation is known to play a key role in the functional regulation of different proteins (Kaleem *et al.*, 2009, 2010). In the current study the internal relationship between phosphorylation and *O*-GlcNAc modification in SIRT1 of *Mus musculus* has been investigated *in silico* and compared with their evolutionary status in different orthologs.

Sirtuins are NAD dependent protein deacetylases, and modulate the aging process by Lys deacetylation of other proteins, and alters their activity and stability (Marton *et al.*, 2010). PTM plays a significant role in increasing the level of SIRT1 protein in cycling cells (Chua *et al*., 2005). SIRT1 is phosphorylated by cyclin B/cell cycle dependent kinase (Cdk1), and this process regulates its deacetylase activity which in turn affects cell proliferation. Phosphorylation and acetylation co-exist to regulate the function and localization of SIRT1. Some studies have shown that SIRT1-deficient mouse cells are resistant to replicative senescence (Chua *et al.*, 2005). Furthermore in aging and senescence, SIRT1 regulated by Cdk1 was shown to control cell proliferation (Sasaki *et al.*, 2008).

As protein modifiers, PTMs function in the same manner in orthologs from phylogenetically related organisms. The multiple alignment of SIRT1 (Fig. 1) shows that all orthologs have very high similarity (amino acid 160-508). The potential phosphorylation sites were predicted as following; 16 sites were conserved (C), 5 were non conserved (NC), and 6 showed a conservative substitution (CS). In mammals however, 5 residues were C and 1 CS, 4 residues were positively predicted yin yang sites (3 Yin Yang sites were C among different orthologs and 1 was CS in mammals only) (Table I). Ser 154, 649, 651 and 683 are potential false-negative Yin Yang sites as these are fully conserved (except Ser 154, which is conserved in mammals, amphibians and birds, Fig. 1), and they showed a potential for phosphorylation and *O*-GlcNAc modification very close to the threshold. This suggests that an interplay between glycosylation and phosphorylation on these sites may regulate the deacetylation activity of SIRT1.

The 4 potential false-negative phosphorylation sites (Ser 154, 649, 651 and 683) have been experimentally determined by Kang *et al.* (2009). They also showed that CK2 phosphorylates SIRT1 on these sites when exposed to ionizing radiation in mouse (Kang *et al.*, 2009). Furthermore, phosphorylation of these residues in SIRT1 was investigated and showed to increase substrate-binding affinity with increasing deacetylation. Another example of SIRT1's ubiquitous activity is in response to DNA damage. When p53 is acetylated on Lys 382 by histone acetyltransferase p300 it increases its affinity towards DNA, whereas deacetylation of p53 by SIRT1 inhibits its affinity (Gu and Roeder, 1997; Sakaguchi *et al.*, 1998; Kang *et al.*, 2009).

SIRT1 also deacetylates the transcription factors FOXO3 and/or FOXO4, which leads to attenuation of FOXO-induced apoptosis and cell-cycle arrest **(**Giannakou and Partridge, 2004). When mammalian SIRT1 deacetylates the transcription factor FOXO4 in a NAD-dependent manner, it increases its transactivation process (Kobyashi *et al*., 2005). SIRT1 is glycosylated through the hexoseamine signaling pathway during starvation or feast conditions (Hanover *et al*., 2010). SIRT1-dependent gluconeogenesis is not regulated by classical gluconeogenic regulatory hormones such as glucagon or insulin, but instead it is mediated by changes in the levels of NAD and pyruvate (Rodgers *et al.*, 2005). *O*-GlcNAc addition opposes the action of SIRT1, on protein function. For example, the key glucagon-responsive transcription factor CREB-regulated transcriptional coactivator 2

Table I.- The potential predicted and experimentally known PTM sites of phosphorylation, glycosylation, Yin Yang sites and False Yin Yang sites along with their conservation status and amino acid residue.

Residue position	Amino acid	O-GlcNAc	Phosphorylation	Yin Yang sites	False Yin Yang sites	Conservation status
14	S	-	+	-	-	DG
25	S	-	+	-	-	NC
46	S	-	+	-	-	DG
82	S	-	+	-	-	DG
151	S	-	+	-	-	CS
154	S	-	+	-	+	NC (Fishes), C (Mammals, birds, amphibians)
160	T	+	-	-	-	CS
161	S	+	+	+	-	C
164	S	-	+	-	-	C
165	S	-	+	-	-	C
169	T	-	+	-	-	CS
234	T	-	+	-	-	C
257	S	+	+	+	-	C
272	Y	-	+	-	-	C
293	Y	-	+	-	-	C
325	S	-	+	-	-	NC
434	S	-	+	-	-	C
446	S	+	-	-	-	C
503	T	-	+	-	-	C
530	S	-	+	-	-	DG
531	S	-	+	-	-	NC
532	S	-	+	-	-	NC
565	S	-	+	-	-	NC
576	T	-	+	-	-	DG
596	S	+	+	+	-	CS(Mammals)
605	S	-	+	-	-	C(Mammals)
609	T	-	+	-	-	C(Mammals)
624	S	-	+	-	-	C
632	Y	-	+	-	-	CS
640	Y	-	+	-	-	C
648	Y	-	+	-	-	CS
649	S	-	+	-	+	C
651	S	-	+	-	+	C
657	S	+	-	-	-	CS
658	S	+	+	+	-	C
663	S	-	+	-	-	CS
665	S	-	+	-	-	C
667	S	-	+	-	-	NC
674	S	-	+	-	-	C (Mammal, amphibian)
683	S	-	+	-	+	C
696	T	-	+	-	-	DG
734	S	-	+	-	-	CS
737	S	+	-	-	-	C(Mammals)

(CRTC2) is responsible for the transcriptional activation of gluconeogenic genes such as signal transducer and activator of transcription 3 (STAT3), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase) and peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) (Gaudy *et al.*, 2010; Hanover *et al.*, 2010; Jitrapakdee *et al.*, 2012). CRTC2 is critical for hormonal signals of glucagon

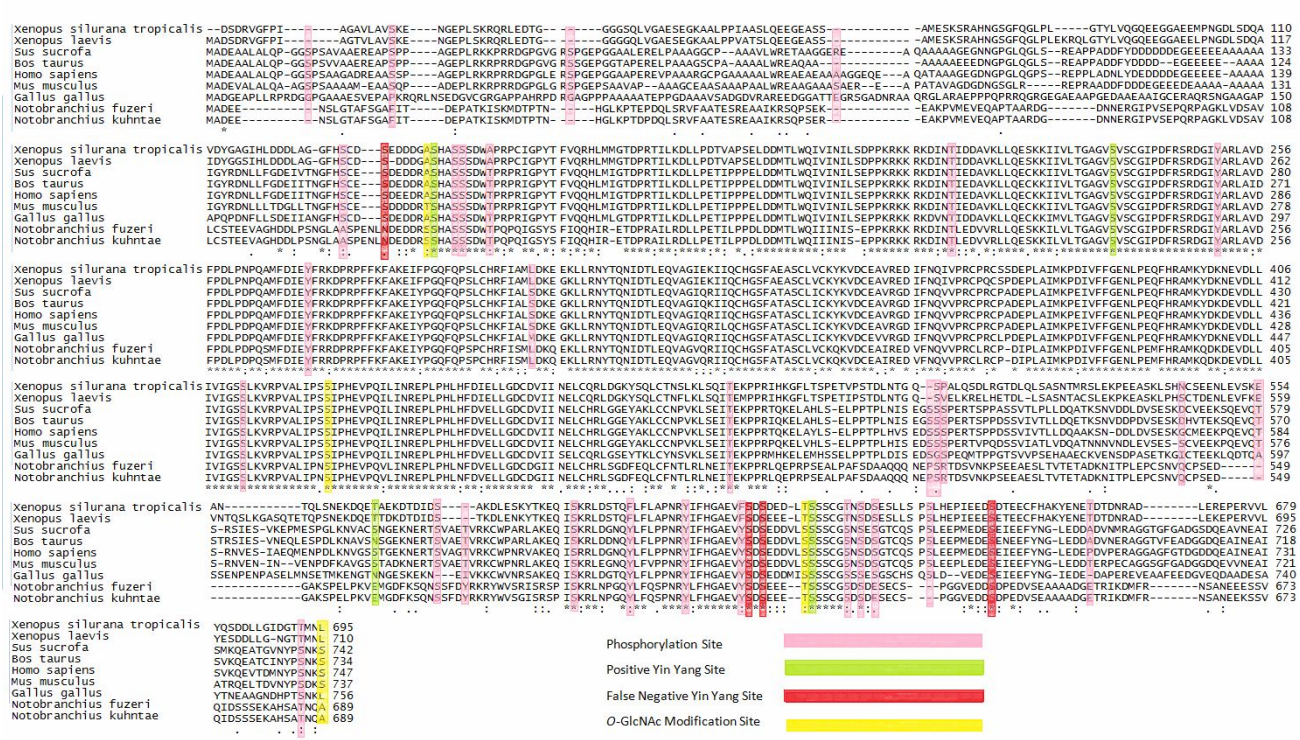

Fig. 1. Multiple sequence alignment of 4 Mammalian, 1 avian, 2 amphibian and 2 fish species using Clustalw2. The arrow sign on the top indicates Phosphorylation sites. The circle shows the potential Yin Yang sites while star on the highlighted line is used for the false negative Yin Yang sites. The rhombus shape signifies O-GlcNAc modified sites. Asterisks show conserved status, double dots show conserved substitution while single dot shows semi conserved/non conserved status.

and insulin in the liver (Yoon *et al.*, 2010). Hyperglycemic phenotype in rodent models of type 2 diabetes is produced by the increased activation of CRTC2-dependent transcription (Dentin *et al.*, 2007) (Fig. 2). Metformin is an anti-diabetic drug that controls hyperglycemia through inhibition of gluconeogenesis (Caton *et al.*, 2010). SIRT1 inhibition partially blocks the effects of metformin on gluconeogenesis (Fig. 3). CRTC2 is *O*-GlcNAc modified, at sites normally phosphorylated to keep CRTC2 in the cytoplasm. Decreasing amounts of *O*-GlcNAc on CRTC2 blocks the effects of glucose on gluconeogenesis by over expression of *O*-GlcNAcase (Dentin *et al.*, 2008) (Fig. 2). Disrupting SIRT1 activity, by liver-specific conditional knockout of the SIRT1 gene or by administration of a SIRT1 antagonist, increased CRTC2 activity and glucose output, and SIRT1 overexpression has the opposite effect (Liu *et al.*, 2008). When p300 acetylates hepatic CRTC2 it increases glucose production, whereas during late fasting, CRTC2 is down regulated by SIRT1 (Hanover *et al.*, 2010) (Figs. 2, 3). This suggests that SIRT1 blocks gluconeogenesis by *O*-GlcNAc modification on Ser/Thr (Fig. 3).

The enzyme endothelial nitric oxide synthase (eNOS) is regulated by SIRT1 in Type I diabetes, binds *O*-GlcNAc at a critical the PKB/AKT site (Ser 177) that is normally phosphorylated to activate

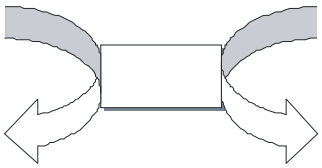

Fig. 2. Function of CRTC2. The P300 protein acetylates CRTC2 to stimulate the glucose production while *O*-GlcNAcase blocks the effects of glucose on gluconeogenesis.

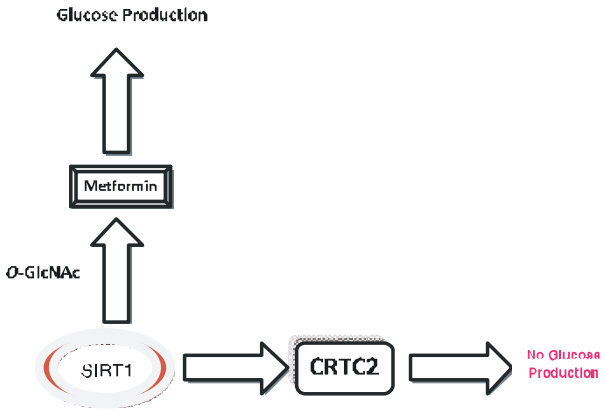

Fig. 3. Effect of SIRT1 on CRTC2 and Metformin. SIRT1 deacetylases CRTC2 during late fasting and CRTC2 is down regulated. Removing SIRT1 increases the activity of CRTC2. Excessive production of glucose due to active CRTC2 initiates Metformin's action and blocks the production of glucose. If SIRT1 is inhibited, Metformin activity on gluconeogenesis is also repressed resulting in over production of glucose.

eNOS (Hanover *et al.*, 2010). *O*-GlcNAc presence at this site is associated with decreased nitric oxide production in diabetic patients (Du *et al.*, 2001; Musicki *et al.*, 2005). SIRT1 deacetylates eNOS, leads to stimulation of eNOS activity and increases endothelial nitric oxide. These studies suggest that OGT-mediated glycosylation and sirtuin-dependent deacetylation may play opposing roles in situations of nutrient excess or starvation (Hanover *et al.*, 2010).

The interplay among different PTMs functionally modulates the proteins that regulate the transcriptional activity of various genes and is altered in pathological conditions. Further studies on Sirtuin will provide better therapies to improve age-associated changes, such as diabetes, cancer and cardiovascular disease, and possibly it can extend healthy human lifespan.

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REFERENCES

ALTSCHUL, S.F., MADDEN, T.L., SCHAFFER, A.A., ZHANG, J., ZHANG, Z., MILLER, W. AND LIPMAN D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl. Acids Res.*, **25**: 3389-3402.

ANASTASIOU, D. AND KREK, W., 2006. SIRT1: linking adaptive cellular responses to aging-associated changes in organismal physiology. *Physiology*, **21:** 404–410.

BERDICHEVSKY, A., VISWANATHAN, M., HORVITZ, H.R. AND GUARENTE, L., 2006, *C. elegans* SIR-2.1 interacts with 14–3–3 proteins to activate DAF-16 and extend life span. *Cell*, **125**: 1165–1177.

BLANDER AND GUARENTE, 2004. The Sir2 family of protein deacetylases, *Annu. Rev. Biochem.*,**73**: 417–435.

BLOM, N., GAMMELTOFT, S. AND BRUNAK, S., 1999. Sequence and structure based prediction of eukaryotic protein phosphorylation sites. *J. mol. Biol.*, **294**: 1351–1362.

BOECKMANN, B., BAIROCH, A., APWEILER, R., BLATTER, M.C., ESTREICHER, A., GASTEIGER, E., MARTIN, M.J., MICHOUD, K., O'DONOVAN, C., PHAN, I., PILBOUT, S. AND SCHNEIDER, M., 2003. The SWISS-PROT protein knowledge base and its supplement TrEMBL in 2003. *Nucl. Acids Res.*, **31**: 365-370.

CATON, P.W., NAYUNI, N.K., KIESWICH, J., KHAN, N.Q., YAQOOB, M.M. AND CORDER, R., 2010. Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5. *J. Endocrinol.*, **205**: 97-106.

CHUA, K.F., MOSTOSLAVSKY, R., LOMBARD, D.B., PANG, W.W., SAITO, S., FRANCO, S., KAUSHAL, D., CHENG, H.L., FISCHER, M.R., STOKES, N., MURPHY, M.M., APPELLA, E. AND ALT, F.W., 2005. Mammalian SIRT1 limits replicative life span in response to chronic genotoxic stress. *Cell Metab.*, **2**: 67–76.

DENTIN, R., LIU, Y., KOO, S.H., HEDRICK, S., VARGAS, T., HEREDIA, J., YATES, J. 3RD AND MONTMINY, M., 2007. Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. *Nature*, **449**: 366–369.

DENTIN, R., HEDRICK, S., XIE, J., YATES, J.R. AND MONTMINY, M., 2008. Hepatic glucose sensing via the CREB coactivator CRTC2. *Science*, **319**: 1402–1405.

DU, X.L., EDELSTEIN, D., DIMMELER, S., JU, Q., SUI, C. AND BROWNLEE, M., 2001. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *J. clin. Invest.*, **108**: 1341–1348.

GAUDY, A.M., CLEMENTI, A.H., CAMPBELL, J.S., SMRCKA, A.V. AND MOONEY, R.A., 2010. Suppressor of cytokine signaling-3 is a glucagon-inducible inhibitor of PKA activity and gluconeogenic gene expression in hepatocytes. *J. biol. Chem.*, **285:**

41356-41365.

GIANNAKOU, M. E. AND PARTRIDGE, L., 2004. The interaction between FOXO and SIRT1: tipping the balance towards survival. *Trends Cell Biol.*, **14:** 408-412

GRAY, S.G. AND EKSTROM, T.J., 2001. The human histone deacetylase family. *Exp. Cell Res.*, **262**: 75–83.

GU, W. AND ROEDER, R.G., 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell*, **90:** 595–606.

GUARENTE, L. AND PICARD, F., 2005. Calorie restriction-the SIR2 connection. *Cell*, **120**: 473–482.

GUPTA, R. AND BRUNAK, S., 2002. Prediction of glycosylation across the human proteome and the correlation to protein function. *Pac. Symp. Biocomput.*, 7**:** 310–322.

HAIGIS, K.M., WISTUBA, I.I. AND KURIE, J.M., 2007. Lung premalignancy induced by mutant B-Raf, what is thy fate? To senesce or not to senesce, that is the question. *Genes Dev.*, **21:** 361-6.

HAIGIS, M.C. AND GUARENTE, L.P., 2006. Mammalian sirtuins-emerging roles in physiology, aging, and calorie restriction. *Genes Dev.*, **20**: 2913–2921.

HANOVER, J.A., KRAUSE, M.W. AND LOVE, D.C., 2010. The hexosamine signaling pathway: O-GlcNAc cycling in feast or famine. *Biochim. biophys. Acta*, **1800:** 80-95.

HIPKISS, A.R., 2006a. On the mechanisms of ageing suppression by dietary restriction-is persistent glycolysis the problem? *Mech. Ageing Dev.*, **127**: 8–15.

HIPKISS, A.R., 2006b, Accumulation of altered proteins and ageing: causes and effects. *Exp. Gerontol.*, **41**: 464–473.

IMAI, S., ARMSTRONG, C.M., KAEBERLEIN, M. AND GUARENTE, L., 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature*, **403:** 795-800.

JITRAPAKDEE, S., 2012. Transcription factors and coactivators controlling nutrient and hormonal regulation of hepatic gluconeogenesis. *Int. J. Biochem. Cell Biol.*, **44:** 33-45.

KALEEM, A., HOESSLI, D.C., HAQ, I.U., WALKER-NASIR, E., BUTT, A., IQBAL, Z., ZAMANI, Z., SHAKOORI, A.R. AND NASIR-UD-DIN, 2011. CREB in long-term potentiation in hippocampus: role of post-translational modifications-studies *in silico*. *J. cell Biochem.*, **112:** 138-146.

KALEEM, A., AHMAD, I., HOESSLI, D.C., WALKER-NASIR, E., SALEEM, M., SHAKOORI, A.R. AND NASIR-UD-DIN, 2009. Epidermal growth factor receptors: function modulation by phosphorylation and glycosylation interplay. *Mol. Biol. Rep.*, **36:** 631-639.

KALEEM, A., AHMAD, I., SHAKOORI, A.R. AND NASIR-UD-DIN, 2008. Regulation of neurofibromin by post-translational modification. *Pakistan J. Zool.*, **40:** 417-422.

KANG, H., JUNG, J.W., KIM, M.K. AND CHUNG, J.H., 2009. CK2 is the regulator of SIRT1 substrate-binding affinity, deacetylase activity and cellular response to DNA-damage. *PLoS One*, **4:** e6611.

KOBAYASHI, Y., FURUKAWA-HIBI, Y., CHEN, C., HORIO, Y., ISOBE, K., IKEDA, K. AND MOTOYAMA, N., 2005. SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress. *Int. J. mol. Med.*, **16**: 237-243

LEIBIGER, I.B. AND BERGGREN, P.O., 2006, Sirt1: a metabolic master switch that modulates lifespan. *Nat. Med.*, **12:** 34–36.

LEVINE, A.J., HU, W. AND FENG, Z., 2006, The P53 pathway: what questions remain to be explored? *Cell Death Differ*., **13:** 1027–1036.

LIN, S.J. AND GUARENTE, L., 2003. Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. *Curr. Opin. Cell Biol.*, **15:** 1–6.

LIU, Y., DENTIN, R., CHEN, D., HEDRICK, S., RAVNSKJAER, K., SCHENK, S., MILNE, J., MEYERS, D.J., COLE, P., YATES, J. 3RD, OLEFSKY, J., GUARENTE, L. AND MONTMINY, M., 2008. A fasting inducible switch modulates luconeogenesis via activator/coactivator exchange. *Nature*, **456:** 269–273.

LONGO, V.D. AND KENNEDY, B.K., 2006. Sirtuins in aging and age-related disease. *Cell*, **126:** 257–268.

LONGO V., 2009. Linking sirtuins, IGF-I signaling and starvation. *Exp. Gerontol.*, **44:** 70–74.

LUCHSINGER, J.A., 2006. A work in progress: the metabolic syndrome. *Sci. Aging Knowledge Environ.*, **2006:**pe19.

MARTON, O., KOLTAI, E., NYAKAS, C., BAKONYI, T., ZENTENO-SAVIN, T., KUMAGAI, S., GOTO, S. AND RADAK, Z., 2010. Aging and exercise affect the level of protein acetylation and SIRT1 activityin cerebellum of male rats. *Biogerontology*, **11:** 679-686.

MICHAN, S. AND SINCLAIR, D., 2007. Sirtuins in mammals: insights into their biological function. *Biochem. J.*, **404:**1–13.

MUSICKI, B., KRAMER, M.F., BECKER, R.E. AND BURNETT, A.L., 2005, Inactivation of phosphorylated endothelial nitric oxide synthase (Ser-1177) by O-GlcNAc in diabetes-associated erectile dysfunction. *Proc. natl. Acad. Sci. USA*, **102:** 11870–11875.

NASIR-UD-DIN, KALEEM, A., IQBAL, Z. AND SHAKOORI, A.R., 2010. The *in silico* interplay between post-translational modification in histone H4 and its role in mouse spermatognesis. *Pakistan J. Zool*., **42:** 787-794.

PILLARISETTI, S., 2008. A review of Sirt1 and Sirt1 modulators in cardiovascular and metabolic diseases. *Rec. Pat. Cardiovasc. Drug Discov.*, **3:** 156–164.

RODGERS, J.T., LERIN, C., HAAS, W., GYGI, S.P., SPIEGELMAN, B.M. AND PUIGSERVER, P., 2005. Nutrient control of glucose homeostasis through a complex of PGC-1a and SIRT1. *Nature*, **434:** 113–118.

SAKAGUCHI, K., HERRERA, J.E., SAITO, S., MIKI, T., BUSTIN, M., VASSILEV, A., ANDERSON, C.W. AND APPELLA, E., 1998. DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev.*, **12:** 2831–2841.

SASAKI, T., MAIER, B., KOCLEGA, K.D., CHRUSZCZ, M., GLUBA, W., STUKENBERG, P.T., MINOR, W. AND

SCRABLE, H., 2008. Phosphorylation regulates SIRT1 function. *PLoS One*, **3:** e4020.

SCHONEICH, C., 2006. Protein modification in aging: an update. *Exp. Gerontol.*, **41:** 807–813.

TANNER, K.G., LANDRY, J., STERNGLANZ, R. AND DENU, J.M., 2000. Silent information regulator 2 family of NAD-dependent histone/protein deacetylases generates a unique product, 1-O-acetyl-ADP-ribose. *Proc. natl. Acad. Sci. USA*, **97:** 14178-14182.

THOMPSON, J.D., HIGGINS, D.G. AND GIBSON, T.J., 1994, CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.*, **22:** 4673-4680.

WALSH, C., 2006, *Posttranslational modification of proteins: Expanding nature's inventory*. Roberts and Co. Publishers, Englewood, Colo, pp. 490.

WESTPHAL, C.H., DIPP M.A. AND GUARENTE, L., 2007. A therapeutic role for sirtuins in diseases of aging? *Trends Biochem. Sci.*, **32:** 555–560.

WU, Y., LI, X., ZHU, J.X., XIE, W., LE, W., FAN, Z., JANKOVIC, J. AND PAN, T., 2011. Resveratrol-activated AMPK/SIRT1/autophagy in cellular models of Parkinson's disease. *Neurosignals*, **19:** 163-174.

YAMAMOTO, H., SCHOONJANS, K. AND AUWERX, J., 2007. Sirtuin functions in health and disease, *Mol. Endocrinol.*, **21:** 1745–1755.

YOON, Y.S., LEE, M.W., RYU, D., KIM, J.H., MA, H., SEO, W.Y., KIM, Y.N., KIM, S.S., LEE, C.H., HUNTER, T., CHOI, C.S., MONTMINY, M.R. AND KOO, S.H., 2010. Suppressor of MEK null (SMEK)/protein phosphatase 4 catalytic subunit (PP4C) is a key regulator of hepatic gluconeogenesis. *Proc. natl. Acad. Sci. USA*, **107:** 17704-17709.

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