The Role of Sirt3 on Age-Related Diseases

By Julie Yang

Age-associated diseases such as cardiovascular disease, cancer, arthritis, diabetes, Alzheimer's and age-related hearing loss (also known as presbycusis) are becoming more prevalent in today's society. These incidences increase more rapidly with aging. The major cause of aging has been associated with the accumulation of oxidative stress.[1] Recent studies have shown that sirtuin 3 (sirt3) activity in metabolism can protect against age-related diseases and is capable of preventing the buildup of oxidative stress. Sirt3 belongs to the third class of histone deacetylase ($HDAC_S$), which are enzymes that cleave off acetyl groups from acetyl-lysine residues in histones. HDACs are important enzymes because they help regulate gene expression. Compared to the other classes of HDACs, sirtuins are unique due to their dependency on nicotinamide adenine dinucleotide (NAD^+) for their catalytic activity. [2]

Sirtuins were first discovered in yeast and can be found in bacteria, plants, invertebrates, and vertebrates.[2] In humans there are seven recognized sirtuins, sirt1-7. These sirtuins are distributed in different areas of the cells, and the reason why sirt3 is an interest of study is because it is located in the mitochondria. The mitochondria play a major role in metabolism and generating reactive oxygen species (ROS). ROS species are formed during metabolism of oxygen. If under environmental stress, ROS levels can increase dramatically and result in cell structure damage. [2]

Sirt3's role in human aging became a topic of interest when the discovery of unique single nucleotide polymorphisms (SNP) were linked to centenarians, people who

have lived beyond age of 100 years. One of the first studies done on sirt3 related to aging showed that in males who carried the G477T transversion in exon 3 of sirt3 were healthy and live beyond the average lifespan.

Several studies have been done that showed sirt3 protecting against ROS production. Some of these findings provide the strongest evidence linking the activity of sirt3 with Caloric restriction (CR). CR has been known to increase lifespan and it is also linked to the activity of sirt3 in reducing ROS and aging. CR is a dietary routine that reduces calorie intake without gaining malnutrition or essential nutrients. Thus, the benefit of CR is that it helps the delay of diseases.[3] One of the disorders associated with aging whose appearance is delayed under CR is age-related hearing loss.

In a study done by Someya et al.[3] the role of sirt3 during CR was investigated. The paper focused on how sirt3 mediates reduction of oxidative stress and prevents age-related hearing loss under CR. Age-related hearing loss (AHL) or presbycusis is the decline in hearing due to aging. In the experiment, a CR diet was imposed onto wild type (WT) and sirt3-/- mice. In wild type mice it was founded that CR slowed down the progression of AHL but not in sirt3-/- mice. Another set of WT and sirt3-/- mice was put under CR to investigate the role of sirt3. Results showed the sirt3-/- mice that were lacking sirt3 gene expression appeared normal during CR. In addition, the study provided an insight into the pathway of sirt3 in response to CR. They suggested that sirt3 directly deacetylates and activates isocitrate dehydrogenase 2 (Idh2), leading to increased NADPH levels. NADPH levels then increase the gluthathione (a biomarker for oxidative stress) ratio. To test out if sirt3 regulates Idh2, the acetylation levels of Idh2 in the mitochondria were measured. The Idh2 levels in WT mice under CR increased in WT

tissues but in sirt3-/- mice CR did not increase Idh2 activity. Further biochemical experiments were done to support the interaction of sirt3 and Idh2. Deacetylation assays were carried out in HEK293 cell, where Idh2 was transfected with and without sirt3. It was isolated by immunoprecipitation with anti-MYC antibody proceeded by Western Blotting with anti-acetyl-lysine antibody. Western Blotting is a technique that allows for the separation and identification of proteins based on molecular weight and antibody binding specificity through gel electrophoresis.[4] Analysis showed that sirt3 deacetylated Idh2 through NAD+ dependent pathway seen by the 100% increased in activity. Thus, protecting the cell against oxidative damage. [3] This studied suggested that under CR, sirt3 deactylates and activates Idh2, which in turn converts NADP+ to NADPH. NADPH helps regenerate reduced glutathione, which protects against ROS, leading to the delay of the progression of AHL.

Similarly in another study, [5] it was found that sirt3 deacetylates and activates the enzyme superoxide dismutase 2 (SOD2). SOD2 is an enzyme located in the mitochondria that plays a role in controlling the levels of ROS. It has been reported that SOD2 has similar effects as that of Idh2. Stimulated SOD2 under CR can increase the levels of NADPH and thus raised the glutathione ratio resulting in a decrease of ROS production. The researchers investigated whether sirt3 deacetylates SOD2. Flag-tagged SOD2 was transfected into 293, immunopurified, followed by Western Blotting with anti-SOD2. Results indicated that sirt3 interacts with SOD2 in vivo. To test what happens under CR diet, the researchers compared acetylation levels of SOD2 activity in WT and sirt3 knockout mice fed control and CR diets. Acetylation levels were measured through immunoprecipitation with anti-SOD2 antibody and analyzed by Western Blotting. It was

found that in WT mice that were fed a CR diet, SOD2 levels were acetylated and deacetylated in CR. However, in sirt3 knockout mice deactylation during CR was not observed. These results are evidence that oxidative stress is decreased when sirt3 activates SOD2. Although, no primary studies have been done to investigate SOD2 in age-related diseases, such as Idh2 with AHL, this new pathway can provide insight into future strategies to prevent diseases.

The role of sirt3 may also be involved in cancer. In a study done by Bell et al, [6] they showed that sirt3 acts as a tumor suppressor via its ability to suppress ROS and regulate hypoxia inducible factor 1α (HIF-1α). HIF-1α is stabilized through ROS. Abnormal stabilization of HIF-1α is associated with various cancers. The researchers examined overexpression of sirt3 under hypoxic conditions. Results showed that the presence of sirt3 diminished the activation of HIF-1α. This reflects the role of sirt3 in ROS production as explained previously. Furthermore, experiments on knockdown of sirt3 in mouse embryo fibroblasts (MEFs) in tumor lines were investigated via quantitative PCR on RNA isolated from cells incubated at O_2 levels. Polymerase chain reaction allows DNA segments to be copied multiple times. In the first cycle of PCR the sample is heated, separating the strands. The temperature is then lower enabling the primers to bind. The taq polymerase synthesizes the complementary DNA strands from free nucleotides. This cycle is then repeated until a multiple segments are produced.[7] In the absence of sirt3 HIF-1α stabilization increased. Thus, in the presence of sirt3 expression HIF-1α stability is reduced and there is a decreased in tumorigensis.

The role of sirt3 in diabetes has also been studied. Opposed from other studies, this paper explains how the reduction of sirt3 expression will increase the levels of

oxidative stress and activate JNK, which stimulates impaired insulin signaling. Jing et al.[8] demonstrated that sirt3 expression in skeletal muscle decreases in type 1 and type 2 diabetes. When the ROS levels increase, it activates Jun-N-terminal kinase (JNK), which phosphorylates the insulin receptor leading to a decrease in tyrosine phosphorylation. This cascade contributes to the development of type 2 diabetes. This decreased in sirt3 activity could possibly contribute to the metabolic abnormalities of diabetes. To investigate these effects, the researchers assayed thiobarbituric acid reactive substances (TBARS) in skeletal muscle in WT and KO mice. There was a 75% increase in TBARS activity in the KO mice, indicating the increase of oxidative stress. Another experiment was done where knockdown cells were treated with 5- and 6-cholromethy-2'-7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA), which monitors ROS concentrations. Results from this showed an increase in ROS levels in the knockdown cells. In conclusion, this study suggested that a decrease in sirt3 expression leads to increased levels of oxidative stress and activation of JNK. This stimulates impaired insulin signaling.

Overall, studies have shown that oxidative stress, which a major factor in aging, can be reduced by the activity of sirt3. In the presence of sirt3 there a high decreased in ROS levels. Sirt3 can combat aging especially by stimulating SOD2 or Idh2 activity during caloric restriction. CR has been shown to prevent age-related hearing loss. Recently, sirt3 as a tumor suppressor has also been studied. Sirt3 has the ability to suppress ROS and regulate (HIF-1α). With more insight into the role of sirt3 in the future, life-threatening diseases can be prevented.

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