Effect of SIRT6 on Smooth Muscle Tissues and Epithelial Tissues- A Review

By

Puja Roy
16346039

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Puja Roy
ID-16346039

Approval

The thesis/project titled “Effect of SIRT6 on Smooth Muscle Tissues and Epithelial Tissues- A Review” submitted by Puja Roy (ID-16346039) of Summer 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons) on 3 February 2021.

Examining Committee:

Supervisor: raushan

Dr. Raushanara Akter

Associate Professor, Department of Pharmacy

Brac University

Program Coordinator: _____

Professor Dr. Hasina Yasmin

Department of Pharmacy

Brac University

Departmental Head: _____

Dr. Eva Rahman Kabir

Professor and Chairperson, Department of Pharmacy

Brac University

Ethics Statement

The study does not involve any kind of animal trial and human trial.

Abstract

SIRT6 is a stress response protein belongs to sirtuin family. It is involved in different multiple molecular pathways that are linked to DNA repair, neurodegeneration, tumorigenesis, glycolysis, gluconeogenesis, cardiac hypertrophy, metabolism etc. The up-regulation and down-regulation of this histone deacetylase involve in different diseases such as Alzheimer's disease, aging, cancer, inflammation, diabetes, and cardiac problems. Thus, it has emerged as an important therapeutic target for the mentioned diseases. This review aims to compile available information regarding SIRT6 and its effect on smooth muscle- and epithelial tissues for predicting future target therapeutic approach. This review information exhibited that increased SIRT6 activity helps to combat diabetic atherosclerotic plaque; heal wound; inhibit EMT; save from pulmonary, cardiac, biliary and renal cell injury; decrease metabolic disease; glioma cell proliferation whereas decreased SIRT6 activity may improve Parkinson's disease. Therapeutic approach can be developed by looking these sorts of effects of SIRT6 in cellular and molecular level.

Keywords: Sirtuin 6; Disease; Smooth muscle tissues; Epithelial tissues; Therapeutic target

Dedication

Dedicated to my parents and my supervisor

Acknowledgement

At first, I want to praise and thank God for the patience, courage he has given me to understand, comprehend, and finish my thesis. Secondly, I am extremely grateful to Dr. Raushanara Akter, Associate Professor at the Department of Pharmacy, Brac University for believing in me, giving me this opportunity, and guiding me along the way with her helpful suggestions without which I would not be able to complete this paper. Thirdly, I would like to offer a huge honor and gratitude to Professor Dr. Eva Rahman Kabir (Chairperson, Department of Pharmacy, Brac University) for her irreplaceable support and care. Lastly, I would really like to thank all my family members and friends for their unfailing love and encouragement and also with utmost respect and appreciation, I wish to extend my sincere gratitude to all the people who have helped me to complete this paper directly or indirectly.

Table of Contents

List of Acronyms

IR	Ischemia/reperfusion
NAD	Nicotinamide adenine dinucleotide
SMCs	Smooth muscle cells
ECs	Endothelial cells
EMPs	Endothelial microparticles
IPF	Idiopathic pulmonary fibrosis
ECM	Excessive extracellular matrix
EMT	Epithelial to mesenchymal transition
AECs	Alveolar epithelial cells
TGF-β	Transforming growth factor-β
RTEC	Renal tubular epithelial cell
TEC	Tubular epithelial cells
AKI	Acute kidney injury
CKD	Chronic kidney disease
VAF	Vascular adventitial fibroblasts
VEC	Vascular endothelial cells
VSMC	Vascular smooth muscle cells
TNF-α	Tumor necrosis factor-alpha

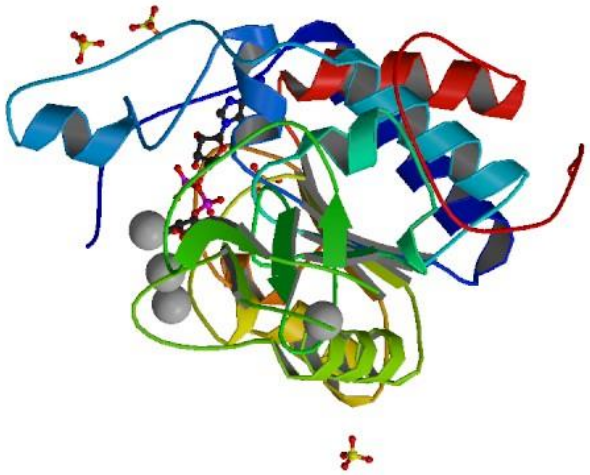
MCP-1	Monocyte chemotactic protein -1
EMT	Epithelial to mesenchymal transformation
AECs	Alveolar epithelial cells
CAD	Coronary Artery Disease
MI	Myocadial infarction
IBEpiC	Intrahepatic biliary epithelial cells
GCDC	Glycochenodeoxycholate
DA	Dopaminergic
HIF-1α	Hypoxia-inducible factor 1-alpha
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
TG	Transgenic
GBM	Glioblastoma multiforme
TDT	Terminal deoxyribonucleotidyl transferase
AIF	Apoptosis-inducing factor
TAC	Transverse aortic constriction
IGF	Insulin-like growth factor
NFAT	Activated T cell nuclear factor
T2DM	Type 2 diabetes mellitus
PPARc	Peroxisome proliferator activated receptor c

Chapter 1: Introduction

1.1 Sirtuin 6

Sirtuins, a family of enzymes are referred as protein lysine deacylases that are Nicotinamide adenine dinucleotide (NAD+)-dependent that can identify cell nutritional levels, modify energy metabolism and stress responses, and are connected to aging processes and diseases associated with aging (You, Zheng, Weiss, Chua and Steegborn, 2019). Sirtuins were first detected in yeasts and subsequent studies in bacteria, plants and animals demonstrated their presence (Szućko 2016). The numerous locations are home to various members of the Sirtuin family. In the nucleus, for example, SIRT1 and SIRT2, as well as the cytosol, SIRT3, SIRT4, and SIRT5 are present in the mitochondria and in the nucleus, SIRT6 and SIRT7 (Khan, Nirzhor & Akter, 2018). Among them, Sirtuin6 (SIRT6) is a stress-response protein that is a SIRT6 gene-encoded mono-ADP ribosyltransferase enzyme (Frye, 2000). It is involved in regulating chromatin and a variety of functions have been shown in metabolism, aging and disease (Khan, Nirzhor & Akter, 2018). Such mammalian sirtuins are involved in gene silencing molecular regulation, DNA repair, lifespan extension, metabolic homeostasis, tumorigenesis, neurodegeneration, and other pathophysiologies of disease (Haigis and Sinclair, 2010). In addition, SIRT6 is closely connected with chromatin known as NAD+-dependent deacetylase H3K9 and H3K566 (Histone H3 lysine 9 and H3 lysine 56, respectively). The enzyme's functions such as telomere maintenance, DNA repair and gene expression are found by studying this histone deacetylation (Khan, Nirzhor & Akter, 2018). Moreover, SIRT6 improves survival in male mice and induces apoptosis and represses aging phenotypes in cancer cells. SIRT6 small molecule modulators are thus used for functional studies and management of aging-related diseases such as cancer and type 2 diabetes (You, Zheng, Weiss, Steegborn and Chua, 2019).

1.2 Structure of SIRT6



Macromolecule Content:

Total Structure Weight: 215.88 kDa

Atom Count: 14021

Residue Count: 1654

Unique protein chains: 1

Figure 1: Crystal structure of human SIRT6 (From Pan et al, 2009)

SIRT6 is composed of two domains named Rossman domain which is the larger one and zinc-binding domain which is smaller one and they consist of the number of eight α-helices and nine β-strands (Pan, P. W. et al., 2011). SIRT6 makes a pocket between these two domains that is like a long hydrophobic-channel and can bind a substrate with an acylated lysine and NAD+. With the action of deacylase, SIRT6 in that pocket moves the acyl group from the substrate to NAD+. Finally, it produces two deacylated substrate product named nicotinamide and 2′-O-acyl-ADP ribose (2′-O-acyl-ADPR) (Pan, P. W. et al., 2011; Jiang, H. et al., 2013).

1.3 Importance of SIRT6

- SIRT6 features great chromatin function in many physiological ways, including the stabilization of telomere and genome, and the repair of DNA (Tennen and chua, 2011).
- SIRT6 helps in homeostasis of glucose, indicating a protective function for metabolic diseases (Xiao et al., 2010; Zhong et al., 2010).
- SIRT6 takes part in the metabolic syndrome, which is characterized by fat and glucose metabolism disorders and also that is considered as an important biomarker for cardiovascular diseases (Mottillo et al., 2010).
- Male mice that overexpress Sirt-6 have longer lifespans and are protected against metabolic disorders caused by obesity (Lombard et al, 2012).
- SIRT6 from human umbilical vein endothelial cells contain anti-inflammatory properties (Lappas, 2012).
- SIRT6 maintain vascular integrity and it protects from oxidative stress-induced damage in vascular smooth muscle cells (SMCs) (Leung, 2016).
- SIRT6 decreases triglyceride synthesis (Li & Kazgan, 2011).

1.4 Rationale of the Study

Since SIRT6 has been shown to be among the most compelling innovative forms of target molecule for affecting various diseases conditions and appears to be evolving to set new targets for potential therapies against these diseases. Researchers have studied the effects of SIRT6 on smooth muscles and epithelial tissues and those studies referred several diversified effects that can mitigate life threatening health issues such as diabetic atherosclerotic plaque, Parkinson's disease, pancreatic cancer, obesity, wound healing etc.

Thus, this review work was carried out in order to gain a better insight and understanding of this comparatively recent therapeutic target for various diseases that are linked to pathogenesis of smooth muscle- and epithelial muscle tissues.

1.5 Aim and Objectives

The aim of this study is to compile available information regarding SIRT6 and understand its effect on smooth muscle- and epithelial tissues for predicting future therapeutic target.

The objectives that led to undertake this study include the following.

- Interpreting a better understanding about the function of sirt6.
- Collecting data about the mechanism of action and pathway of SIRT6 towards disease conditions that are linked to smooth muscle- and epithelial tissues
- Knowing more about the type of association between SIRT6 and the various conditions of the diseases originate from smooth muscle- and epithelial tissues pathogenesis.
- Finding out under what conditions this kind of therapy might be applicable
- Obtaining a better perspective on the potential factors that make the role of SIRT6 therapy better.

Chapter 2: Methodology

This review paper has been prepared by extraction of multiple information from various scientific articles of various journals. The evidence, data and statistical values mentioned in this paper has been extracted from various scholarly articles. After conducting a comprehensive review of a number of articles, the vital information was traced, combined and synchronized accordingly. After going through continuous search, the most relevant articles were selected and examined for pinpoint information. Afterwards, based on the topic, the most accurate information was collected that suites accurately with the topic. Various online search engines and journal databases such as, Google Scholar, ACS Publications, Nature, SpringerLink, Wiley Online Library, PubMed, Science Direct have been used for searching information as per requirement of the topic. The array of articles as per the need of this review paper.

Chapter 3: Role of SIRT6 in different diseases that are caused by pathogenesis of smooth muscle tissues and epithelial tissues

2.1 Diabetic atherosclerotic plaque and endothelial dysfunction

The major cause of death in cases of type 2 diabetes is cardiovascular disease. Diabetes enhances the sensitivity to plaque damage and contributes to increased clinical incident frequency and severity (Balestrieri et al. 2015). Atherosclerosis is one kind of chronic inflammatory disorder that develops through years, causing clinical incidents such as heart attacks and strokes to cause cardiovascular disease, and begins with changed proteoglycans in which lipoproteins are trapped in the vessel wall. (Tabas et al., 2007 & Little et al., 2008). The degradation and dysfunction of the vascular endothelium is observed and it is then followed by a complex inflammatory reaction which involves multiple immune cells and among them, some immune cells facilitate the development of atherosclerotic plaques (Libby, 2002; Little et al., 2011 & Ross, 1999). Plaques may remain in different forms such as stable or labile. Labile plaque can cause many clinical events which are life threatening (Devies, 1996). In the progression of diabetic vascular complication, vascular endothelial function is compromised and it is preferred as the key factor in the complication. This event is showed through inflammation conditions, downregulated blood flow, cell proliferation, decreased development of nitric oxide (NO) and endothelial cell death, which results in high mortality rates (Natali as cited in Jing et al., 2017).

Endothelial cells (ECs) is cultured and different experimental models of DM have recently been used to investigate what are the potential role of endothelial dysfunction in cardiovascular disorders. In this process, endothelial dysfunction actually manifests itself as endothelial degradation, apoptosis, inflammation and marked release of EMP. (Bernard et al., 2009; Chen et al., 2014). Notably, endothelial cells, which include microparticles, exosomes, and apoptotic

bodies, can release membrane vesicles of about 0.1 to 1.0 μm in diameter. Blebbing plasma membrane and endothelial protein wrapping make up these microparticles. Here, endothelial microparticles (EMPs) work as a surrogate biomarker for endothelial dysfunction. TNF-α, ROS, inflammatory cytokines, lipopolysaccharides, thrombin and low shear stress promote the formation of endothelial microparticles (Leopold, 2013; Jing et al., 2017).

Within ECs, nuclear factor-kB (NF-kB) expression gets enhanced with the loss of SIRT6 level. In contrast, NF-kB expressional activity is decreased when SIRT6 gets overexpressed. It suggests that SIRT6 have correlation with the upregulation of genes which are involved in inflammation, vascular dysfunction, and angiogenesis. Moreover, for diabetic patient, the inflammatory conditions of atherosclerotic plaque develop. And then, experimental and clinical studies have already shown that atherosclerosis has connection with reduced number of ECs and its dysfunction (Balestrieri et al., 2015).

Jing et al. used one tool called ELISA to evaluate the plasma SIRT6 level and expression and the result showed that the plasma expression of SIRT6 from DM patients was substantially decreased compared to that of healthy individuals. Furthermore, in endothelial microparticles (EMP), expression of SIRT6 mRNA and SIRT6 protein have also been studied. Unlike SIRT6 mRNA, experiments with immunoblots showed almost no expression of SIRT6 proteins in different EMPs. This means that in EMPs, SIRT6 mRNA is selectively packaged. Finally, in patients with elevated blood glucose levels, EMPs and plasma SIRT6 levels were also measured, showing elevated levels of EMP and decreased SIRT6 levels. Therefore, it is said that this increased blood glucose and decreased SIRT6 increases the release of EMP, which causes endothelial dysfunction, including increased development of inflammatory cytokines.

Consequently, the risk of damaged endothelial cells being examined in DM patients is assessed as an effective source of harmful EMPs. In diabetic patients, the levels of EMPs and glucose-

cultured HUVECs are high, while plasma SIRT6 is low. Inflammatory chemokine release and blunt EC angiogenesis are improved by EMPs induced from diabetic patients. In contrast, SIRT6 mRNA enriched EMPs induce EC angiogenesis, and eNOS phosphorylation is increased and inflammatory chemokine release is delayed. In addition, SiRNA inhibits the expression of SIRT6 mRNA in EMPs, reduces angiogenesis and eNOS phosphorylation, but increases cell inflammation (Jing et al. 2017). Collectively, these findings suggest that SIRT6 plays a vital role in the regulation of endothelial function and that enhanced SIRT6 activity may be a potential therapeutic strategy for treating atherosclerotic disease (Xu et al. 2016).

2.2 Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is one sort of chronic, progressive and restrictive interstitial lung disease that can be marked by severe extracellular matrix (ECM) deposition and degradation of the lung architecture. (Tian et al., 2017). This type of lung disease results in scarring (fibrosis) of the lungs for an unknown reason. The scarring becomes worse over time and it becomes impossible to take in a deep breath and enough oxygen cannot be taken in by the lungs (the lung association, 2018). IPF patients have a short life expectancy after diagnosis which is about 2 to 3 years, as well as the incidence of IPF keeps increasing.

Myofibroblasts are the key effector cells in the initiation and progression of lung fibrosis and it is characterized by increased proliferation of alpha-smooth muscle actin and the development of a large number of ECM components. The reduction of myofibroblast synthesis and its activation has been used as a beneficial clinical tool to combat and treat lung fibrosis (Tian et al., 2017). Myofibroblast aggregation is prone to be caused by three mechanisms: (1) stimulation of resident fibroblasts due to pulmonary injury (Phan, 2002); (2) spreading bone marrow origin of mesenchymal fibrocytes (Moeller et al., 2009); (3) epithelial to mesenchymal transition (EMT) differentiation of lung alveolar epithelial cells (AECs) into myofibroblasts (Kim et al., 2006; Wills et al., 2006). Although a lot of time and effort was already made to

identify this disease's pathogenesis, hardly any effective treatment has been found to extend the life of IPF patients and for this, lung transplantation is the only treatment. So, further analysis of IPF pathogenic mechanisms is therefore necessary to identify new molecular targets which can change or stop the growth of this disease (Zhang et al. 2019).

Fibroblast-to-myofibroblast differentiation is a crucial step in the IPF process, so avoidance of this step is a vital therapeutic strategy. Zhang et al. (2019) found that SIRT6 reduces pulmonary myofibroblast differentiation through minimizing transforming growth factor beta1 (TGF-β1)-induced phosphorylation and associated nuclear translocation of Smad2. Epithelial to mesenchymal (EMT) transformation however is a process in which epithelial cells continue to gain a mesenchymal phenotype by losing epithelial morphology and biomarkers. In addition, in lung biopsy, EMT phenotypes from IPE patients have been commonly observed.

Lung epithelial cells appear to be the main target of lung damage in IPF. In addition to phenotypic changes following chronic lung injury, epithelial cells undergo functional changes identified via synthesis and secretion of a variety of profibrotic variables, which in addition contribute to EMT and IPF. In addition, they demonstrated how induced expression of SIRT6 that almost entirely eliminated TGF-β1-induced migratory behavior. TGF-β1 /Smad3 signaling is involved in SIRT6- mediated EMT inhibition, which is the key signaling mechanism that regulates EMT. Tian et al. (2017) noticed that the anti-EMT effects of SIRT6 are mostly triggered through inactivation of TGF-β1 / Smad3 processing and that is reliant upon the deacetylase action of SIRT6. Moreover, they showed that SIRT6 attenuates EMT-related transcription factors in some kind of a way such as catalyzed action. An important observation of this study seems to be that for both vitro and in vivo EMT phenotypes, SIRT6 is negatively regulated. Interestingly, the EMT process is a reversal biological system which appears to apply to the dedifferentiation of mesenchymal cells to epithelial cells as a cell plasticity mechanism (Thiery et al., 2009).

Therefore, SIRT6 expression will inhibit the differentiation between fibroblast-to-myofibroblast (Zhang et al. 2019) and epithelial to mesenchymal transition (EMT) (Tian et al. 2017), which contributes to the production of basic IPF treatments.

2.3 Wound healing

Skin wound healing is considered as a highly arranged mechanism consisting of several distinct phases: (i) a phase that cause inflammation which mostly clears debris as well as bacteria, (ii) a phase where proliferation occurs and it refills the area of the dermal wound, and (iii) a lengthy remodeling phase that includes inflammation recovery and connective tissue restructuring into yet another scar tissue (Koo et al. 2019).

An early event in the tissue healing cycle is the influx of inflammatory cells through into the the wound site. The first stage of protection against infection is neutrophils, which is the proinflammatory cytokines origin (Kanno et al., 2011). Wound macrophages help to control wound healing by creating different growth factors, including such transforming growth factor-β (TGF-β), basic fibroblast growth factor, and platelet-derived growth factor. In response to these growth factors, epithelial cells begin to grow and move to cover the wound and endothelial cells take part in angiogenesis, and fibroblasts make a substantial contribution to the dermal healing mechanism (Barrientos et al., 2008).

Koo et al. (2019) demonstrated that myeloid cell-specific SIRT6 deficiency contributes to slow down healing process of wound. This abnormal wound closure trend was linked to an increased invasion of macrophages which ended in failure to phenotypically move from M1 to M2 macrophages. Besides, Thandavarayan et al. have previously discovered that SIRT6 knockdown disrupts wound closure of patient with diabetes and it concurrently elevates the rate of oxidative stress, inflammatory cytokines and NF-egB activation throughout skin wounds. Likewise, the sirtuin activator, which is resveratrol accelerates wound healing via

enhancing keratinocyte proliferation, whereas the sirtuin inhibitor named sirtinol delays wound healing.

In addition, recently, the study of Hu et al. revealed that corneal wound healing process is delayed and remains incomplete for SIRT6 knockout mice. These experiment manifests that the demonstration of delayed and unfinished recovery of the cornea performs a potential role for mice following injury. These studies suggest a beneficial role of SIRT6 activation in the healing of wounds upon injury.

Koo et al. (2019) attempted to figure out how a SIRT6 deficiency specific to myeloid cells could actually impact the penetration of macrophages and wound closure utilizing mS6KO mice. Then, their study revealed that excisional wound macrophages is increased in amount markedly in mS6KO mice. The substantially increased development of a macrophage chemoattractant and CCL2 in mS6KO mice may be responsible behind the increased macrophage penetration observed in the wounds of this mice strain compared to wild type mice. Surprisingly, there is clearly shown diversity in the macrophages that is recruited from the wounds of mS6KO mice. Activity rates of known M1-specific marker genes have been upregulated in skin wounds in mS6KO mice, although activity levels of M2 marker genes have been downregulated. These findings suggest that in mS6KO mice, the wound condition supports the proinflammatory M1 status (Koo et al. 2019). They showed using in vivo and in vitro methods that SIRT6 deficiency in myeloid cells uses the more proinflammatory cytokines such as TNF-a, IL-1β, and IL-6 discovered in mS6KO mice. It is well known that M1-type macrophages seem to be an origin of proinflammatory cytokines. The regular performance of epithelial and dermal fibroblast cells is modified by these cytokines and eventually delays the speed of epithelial closure and wound closure or dermal closure (Mahdavian Delavary et al., 2011). Besides, SIRT6 deficiency in myeloid cell has a negative function during excisional

wound healing. Thus, SIRT6 activation specific to myeloid cells may also be a therapeutic strategy for speeding wound healing (Koo et al. 2019).

2.4 Renal tubular epithelial cell injury

The renal tubular epithelial cell (RTEC) is referred as a layer of cells which located in the outer layer of the renal tubule and it performs a vital role throughout the renal system. RTEC helps to absorb different substances like glucose, amino acids, and other substances. At the same time, they have the function of discharging acid, regulating the water-electrolyte balance, regulating the acid-alkali balance function. Injury of renal tubular epithelial cells contributes to irregular secretion of cells, which releases different inflammatory cytokines such as IL-1, CTGF, bFGF, TGF beta, PDGG etc (Cloud-Clone Corp.). Tubular epithelial cells (TEC) also play a central role mostly for progressive renal injury by modifying routes associated with partial epithelial-mesenchymal transformation, cell cycle arrest at G1/S and G2/M sites, as well as metabolic syndrome (Liu et al. 2018).

Acute kidney injury (AKI) is a disease with a high rate of occurrence and mortality (Marx et al., 2018). Sadly, the kidney does not completely recover after AKI, which further develops into chronic kidney disease (CKD) (Basile et al., 2016; Chawla, Eggers, Star, & Kimmel, 2014; Hsu, 2012). In order to prevent its emergence into CKD, it is therefore necessary to recognize possible curative targets.

As a NAD+-dependent deacetylase, various forms of biological processes have been associated with sirtuin 6 (SIRT6). The function of SIRT6 under hypoxic stress has been investigated in tubular epithelial cells (TECs). After generating ischemia/reperfusion (IR) injury and hypoxia-challenged TECs, SIRT6 expression in the mouse kidney was examined. It is studied that SIRT6 influences hypoxia-induced injury along with inflammation and epithelial-to-mesenchymal transition by the use of SIRT6 plasmid and small interfering RNA.

Through an oxygen-deficient situation, the expression of SIRT6 in HK-2 cells is evaluated to examine the effect of hypoxia on SIRT6 expression in vitro. The SIRT6 expression in the nucleus of HK-2 cells was shown to be steadily decreased at different points of time after introduction of hypoxic conditions. Furthermore, western blot analysis showed that at various time intervals, the expression of SIRT6 in HK-2 cells was gradually decreased under hypoxic conditions. These findings revealed that the expression of SIRT6 in HK-2 cells could be decreased by hypoxia. So, in this case, it can be said that the cell cycle has been observed in hypoxia-challenged TECs. Additionally, with IR injury and hypoxia-challenged TECs in the kidney of mice, SIRT6 was downregulated.

Afterward, for hypoxia-induced TECs, the overexpression of SIRT6 in HK-2 cells has been used to monitor the impact of SIRT6 on inflammation levels. Next, the variations in MCP-1 and TNF-alpha expressions in SIRT6-over-expressing cells are examined (Gao et al. 2020). Previous research has suggested that in renal fibrosis, EMT has a crucial role (Bai et al., 2017). Gao et al. (2020) observed that even in hypoxia-challenged HK-2 cells, overexpression of SIRT6 enhances E-cadherin expression, whereas FN and alpha-SMA expressions were inhibited by SIRT6 overexpression, which indicates that SIRT6 preserved HK-2 cells against hypoxia-induced EMT. In TECs, SIRT6 has been shown to collectively inhibit inflammation caused by hypoxia and EMT (Gao et al. 2020).

Consequently, the depletion of SIRT6 aggravated injury that is hypoxia-induced and also G2/M phase arrest occurs. For this, the cell cycle in SIRT6 siRNA transfected HK-2 cells is evaluated to decide if SIRT6 deficiency enhanced G2/M arrest in hypoxia-challenged TECs. Flow cytometry checked the cell cycle under hypoxia in SIRT6-depleted TECs. G2/M phase is arrested which extensively become elevated in SIRT6-depleted HK-2 cells under hypoxic situations in comparison to the control group.

Hypoxia-induced damage and G2/M phase arrest are attenuated in TECs by SIRT6 overexpression. The phase arrest of epithelial cells after damage in the cell cycle G2/M may control renal fibrosis (Yang, Besschetnova, Brooks, Shah, & Bonventre, 2010). The cell cycle protein expressions are analyzed to determine if SIRT6 is associated in the cell cycle monitoring process. Cyclin D expression in phase G1 has been observed and also Cyclin B accumulation is, however, observed to be higher in the G2/M phase. Cyclin B1 is significantly suppressed in HK-2 cells with over-expressed SIRT6 against hypoxic conditions in comparison to the control group. However, no improvements in the expression of cyclin D1 have occurred. Such results suggested that by suppressing G2/M arrest from hypoxia-mediated injury, SIRT6 completely protected HK-2 cells. Likewise, through resisting G2/M phase arrest, SIRT6 inhibited hypoxia-triggered TEC injury. SIRT6 is also a potential candidate for mitigating the symptoms of kidney injury (Gao et al. 2020).

2.5 Inflammation of vascular adventitial fibroblasts

Vascular adventitia, described as the region between the outer elastic lamina and the outer edge of the blood vessel, which mainly consists of fibroblasts and has been regarded as solely a passive supporting structure throughout the blood vessel for years (Wang, 2010). Vascular adventitia plays a crucial role in controlling cardiovascular functions and vascular homeostasis and also leads to the cardiovascular disease progression. Various causes of hypertension, atherosclerosis and vascular damages are characterized by the proliferation, migration and differentiation of adventitial fibroblasts. The function of angiotensin II (ANG II) is well known for the development of hypertension and atherosclerosis. Furthermore, it is understood that ANG II is involved in cardiovascular and renal disease pathogenesis through the control of cell formation, inflammation or fibrosis. ANG II in cultured adventitial fibroblasts promotes the proliferation and migration of adventitial fibroblasts and the growth of myofibroblasts. It has

been well known that ANG II enhances the production of collagen throughout different fibroblasts (Physiology 2015).

He et al. 2017 noticed the presence of SIRT6 in vascular adventitial fibroblasts (VAFs), vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs) which cannot be changed by tumor necrosis factor-alpha (TNF-α). SIRT6 expression was lowered in TNF-alpha-treated VAFs. However, TNF-alpha noticeably increased the expression of monocyte chemotactic protein 1 (MCP-1) and interleukin (IL)-6. The SiRNA knockdown of SIRT1 and SIRT6 greatly enriched TNF-alpha-influenced MCP-1 and IL-6 expression. The overexpression of SIRT1 and SIRT6 inhibit the TNF-alpha-induced expression of MCP-1 and IL-6 in VAFs. Moreover, it is also found that SIRT1 positively regulate the expression of SIRT6 in VAFs. In addition, the knockdown of SIRT1 and SIRT6 respectively increased the generation of reactive oxygen species (ROS) and phosphorylation of protein kinase B (Akt) induced by TNF-alpha. Oxidative stress is an underlying cause of vascular disorders, including inflammation, hypertension and atherosclerosis. SIRT6 expression in vascular smooth muscle cells (SMCs) can provide protection against those oxidative stress related damage. To research the role of SIRT6 in SMCs, a novel strain of SMC-specific SIRT6-deficient (SIRT6KO) mice has been created with Cre-lox technology. Angiotensin II (Ang II) was then injected to induce oxidative stress since no other abnormalities were found in the aortas of the SIRT6KO mice. SIRT6KO mice acquired different problems like aortitis, aortic hemorrhage, and aneurysms in exposure to Ang II. SIRT6 therefore plays an anti-inflammatory function in aortic SMCs, which is essential to preserve the integrity of the vessel wall in the context of oxidative stress (Leung 2016).

2.6 Myocardial infarction (MI)

Coronary artery disease (CAD) is referred to as a very familiar complicated disease induced by atherosclerosis, an immune metabolic disease. Family history, age, smoking, hypertension, obesity, diabetes, hyperlipidemia and hypercholesterolemia are some risk factors that are well known. Many other similar genome-wide studies on CAD have been performed and more than 50 genetic variants of genome-wide relevance have been correctly identified. Here however, these fields together contribute CAD cases about 10% (Deloukas et al., 2013; Roberts, 2014; Bjo¨rkegren et al., 2015; Ozaki and Tanaka, 2015). The major factors of CAD have currently been indicated the genetic variants with low and rare frequencies and myocadial infarction (MI). Study demonstrated that epigenetic variables also lead to a fraction of CAD cases (Nu¨hrenberg et al., 2014).

Via controlling gene expression and protein activities, SIRT6 plays different roles in genomic integrity, metabolism of glucose and lipid, stress tolerance, and expected life span. In addition, SIRT6 is already clinically connected with cardiovascular diseases, diabetes, obesity, cancer, and inflammation and so on (Tennen and Chua, 2011; Etchegaray et al., 2013; Kugel and Mostoslavsky, 2014; van Meter et al., 2014). Mostoslavsky et al., (2006) studied that SIRT6 gene knockout mice seems to develop cardiovascular disease seen in animal studies for the first 2 weeks and die at about 1 month of age, likely due to rapid aging and hypoglycemia. A new survey demonstrates that SIRT6 influences the fate of embryonic stem cell in mice (Etchegaray et al., 2015). Additionally,human and animal studies have already indicated that SIRT6 provides protective effects on cardiovascular of cardiovascular disorders such as cardiac hypertrophy, ischemia-reperfusion and heart failure (Winnik et al., 2015). SIRT6 prevents the production of cardiac hypertrophy by actively controlling insulin growth factor (IGF)-Akt signaling (Sundaresan et al., 2012; Pillai et al., 2014). Akt signaling is considered as to control normal development of the heart, contractile function, and coronary angiogenesis (Shiojima

and Walsh, 2006). SIRT6 mediates the defensive function of hypertrophic nicotinamide mononucleotide adenylyl transferase in animal models and suppresses cardiomyocyte hypertrophy by inhibiting the kappa-light-chain-enhancer activated B cell nuclear factor (NF-kB) (Caietal., 2012; Yuetal.,2013). During ischemia-reperfusion, SIRT6 safeguards the heart against apoptosis (Cattelanetal., 2015). In addition, overexpressed SIRT6 prevents hypoxic damage to cardiomyocytes (Maksin-Matveevetal., 2015). SIRT6 even restricts differentiation from cardiac fibroblast into myofibroblast (Tian et al.,2015; Wang et al. 2016).

In patients with heart failure, myocardial testing showed a significant decrease in the amount of SIRT6, indicating that SIRT6 signaling plays a critical part in heart disease. Therefore, since SIRT6 is predominantly expressed in the heart, it can be considered as an important modulator of effective cardiovascular functions and diseases.

2.7 Apoptosis of human intrahepatic biliary epithelial cells (HiBEC)

Intrahepatic biliary epithelial cells (IBEpiC) line, a complex three-dimensional network of tubular ducts with varying diameters in the liver. They constitute just 3-5 % of the total liver cell population, but generate as much as 40% of the bile's daily production. A number of studies have shown that, through a coordinated sequence of hormone-regulated secretory and absorptive processes, IBEpiC performs a vital role in sustaining, changing and increasing the canal bile composition. IBEpiC also plays an active immunologic role for both innate and adaptive immune systems. IBEpiC secretes chemokines and cytokines and expresses essential cell adhesion molecules to localize the immune response (ScienCell).

Biliary epithelial cells and hepatocytes are also introduced to toxic bile acids resulting from homeostasis of bile acid disruption in their microenvironment in cholestatic liver disease (Yang et al., 2019). Hydrophobic bile acids are strongly cytotoxic, such as glycochenodeoxycholate (GCDC), which can induce both hepatocyte and bile duct epithelial cell apoptosis. The

mechanism of bile acid-induced bile duct epithelial cell injury must, however, be addressed both to mitigate biliary and liver damage and to aid in the creation of new biomarkers (Zhang et al., 2018).

In several organs, including the liver, SIRT6 has shown to play a significant role for defending against ischemia/reperfusion damage (Zhang et al., 2018). Following data has been found by Li et al. 2020,

- GCDC mediated SIRT6 expression down-regulation in HiBEC.
- The GCDC-induced HiBEC apoptosis was strengthened by SIRT6.
- SIRT6 questioned the GCDC-induced mtDNA injury.
- SIRT6 mediated activation of PGC-1 Al via the AMPK pathway.

In recent years, scientists have synthesized and tested pyrrolo[1,2-a] quinoxaline derivatives and acquired the very first synthetic SIRT6 activator (You et al., 2017). Moreover, SIRT6 is commonly involved with ischemia/reperfusion, alcoholic liver damage, cardiovascular disorder, renal disease, and hepatocellular carcinoma (Kim et al., 2019; D'Onofrio et al., 2018; Liu et al., 2017; Huang et al., 2018; Zhao et al., 2018). The HiBEC apoptosis caused by bile acid could be decreased by SIRT6. In this way, SIRT6 could become a new gene target for the cholestatic liver disease treatment. Further research on SIRT6 may offer new hope for cholestasis therapy (Li et al. 2020).

2.8 Parkinson's disease

Parkinson's disease (PD) is an age-related neurodegenerative state that is characterized by progressive death of dopaminergic neuron (DA) and which leads to muscle impairment, behavioral changes, and often dementia. There is no medication to prevent neuronal cell death or pause PD progression (Obeso et al., 2010). Several genetic medical conditions, including

such mutations in alpha-Synuclein, LRRK2, and Parkinin, have been reported in inherited cases of Parkinson's (Dawson, 2000). Observational surveys have identified many factors that raise PD occurrence, including the exposure to herbicides, certain dairy products, traumatic brain injury, or being overweight. Surprisingly, government-sponsored health researches show that smoking reduced PD deaths by 64 percent in 1959, from 200,000 veterans in the United States. In contrast, the tobacco component nicotine is presumed to be a major neuroprotection mediator (Bencherif, 2009; Pfister et al., 2008). So, the protective role of PD with tobacco and nicotine remains an open question, but research into this phenomenon created a chance to identify new therapeutic targets.

SIRT6 has always been part of the NAD+-dependent enzyme sirtuin family, which has become a focus for age-related disorders, including neurodegeneration (Herskovits & Guarente, 2013). SIRT6 inhibitors and activators are being treated for a wide range of diseases, but SIRT6 has not really been studied in the PD context before. SIRT6 activity promotes apoptosis in multiple cell types (Van Meter et al., 2011). Thus, for some cancers, its activation is indicated to be advantageous (Sebastian et al., 2012). However, in non-cancer cells, including neurons, SIRT6 activity can also promote apoptosis (Cardinale et al., 2015). In addition, recently, inhibition of SIRT6 has also been shown to inhibit stress-induced apoptosis and to protect against retinal neurodegeneration. SIRT6 promotes inflammatory cytokine development and discharge of it. And so, chronic inflammation is thought to be the basis of neuronal death in PD and as well as neurodegenerative diseases. It has been shown that the risk preventive factor for PD, cigarette smoke, decreases SIRT6 abundance in human lungs and cell culture, while positive risk factors such as paraquat overabundance and fatty acids both increase the development of SIRT6. In research, overexpression of SIRT6 has been reported to increase the survival of mice and improve some age-related diseases in rodents. Based on this reasoning, SIRT6 should protect from several age-related diseases, including PD. However, even at an advanced age, rodents

do not naturally grow PD. Based on proven SIRT6 functions, SIRT6 activity can have a distinct effect on ageing related human diseases, and requires a thorough analysis of the connection among SIRT6, neurodegeneration, and PD environmental risk factors (Nicholatos et al. 2018).

2.9 Tumor angiogenesis of lung cancer

Lung cancer has become a single largest cause of cancer deaths in the U.S. and globally, both in men and women (MedicineNet). Cancer has the capacity, which makes it life-threatening, to spread to adjacent or distant organs. Tumor cells have the ability to invade the blood or lymph vessels, spread throughout the intravascular system, and afterwards metastasize in another location (Folkman 1971). For such metastatic spread of cancer tissue, proliferation of the vascular network seems to be critical. These procedures are referred to as angiogenesis by which new blood vessels develop. It certainly plays an important role in the creation of a new vascular network due to the distribution of nutrients, oxygen and immune cells and also the disposal of waste products (Folkman 1971).

Both activator and inhibitor molecules control angiogenesis and they have been identified as a variety of different proteins. The expression rates and levels of angiogenic factors reflect the aggressiveness of tumor cells (Nishida et al., 2006). SIRT6 overexpression may lower the amount of angiogenic factors. Hypoxia-inducible factor 1-alpha (HIF-1α) plays a significant role in fostering angiogenesis. The study of Wang, Sheng, and Cai (2018) shows that after inhibiting HIF-1 alpha, SIRT6 can lower the level of the vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR). In addition, the results of the xenograft research showed that overexpression of SIRT6 also inhibited HIF-1 alpha expression and decreased VEGF level, which agrees with the results of the cell experiment that overexpression of SIRT6 inhibited xenograft angiogenesis, suggesting that SIRT6 has anti-angiogenesis effects which can be a possible target to treat the lung cancer. Furthermore, it is also found that overexpression of SIRT6 in cell line A549 can lower the level of HIF-1 alpha, which suggests

that through HIF-1 alpha, SIRT6 can regulate the metabolism of tumor energy and with SIRT6-deficient cells show a decrease in mitochondrial respiration in increased HIF1 alpha activity and glycolytic glucose uptake (Wang, Sheng, and Cai 2018). Thus, in near future, SIRT6 can perform as an effective treatment option for lung cancer treatment.

2.10 Obesity

A dynamic disease involving an excessive and unhealthy amount of body fat is obesity. Obesity is not really just the cosmetic issue. This is a medical condition which carries the potential risk of other illnesses and health conditions (MAYO CLINIC). One of the 21st century's big epidemics is metabolic syndrome. This disorder comprises several category of disorders that have included abdominal obesity, dyslipidemia, sensitivity to glucose, insulin resistance, and early onset of age-related diseases such as type II diabetes, hypertension, generalized inflammation, and a propensity to generate neurodegenerative diseases (Grundy et al., 2005; Milionis et al., 2008; Huang, 2009). For adults, overweight and obesity are defined by WHO as below:

• Overweight: the BMI is greater than or equal to 25; and

• Obesity: The BMI is equal to or greater than 30.

The NAD+-dependent SIRT6 deacetylase represents as a preventive candidate for the growing outbreak metabolic disorder. SIRT6 deficiency in mice give rise to early aging phenotypes and metabolic abnormalities and this situation happened in a calorie restriction experiment showing a reverse collection of metabolic syndrome phenotypes (Kanfi et al. 2010). The calorie restricted (CR) diet tends to decrease the aging rate and increase the life span of many animals, including yeast and rodents and it is well known for more than 70 years (McCay et al., 1935; Weindruch & Walford, 1988). In order to better understand the SIRT6's role in metabolic SIRT6 overexpressed wild type and transgenic (TG) mice were given a high fat diet. Compared

to the wild-type littermates, SIRT6 TG mice developed significantly less visceral fat, LDL-cholesterol, and triglycerides. Besides, TG mice displayed enhanced acceptance of glucose along with increased glucose-induced insulin secretion. Besides, examination of the expression of the adipose tissue gene has showed the greater impact of overexpression of SIRT6 and that is also associated with the downregulation of a selective set of peroxisome proliferator-activated receptor-responsive genes and lipid storage-related genes, such as angiopoietin-like protein 4, adipocyte fatty acid-binding protein, and diacylglycerol acyltransferase. These results show that SIRT6 has a protective role towards the metabolic effects of diet-induced obesity and indicate that activation of SIRT6 could provide a positive influence on age-related metabolic diseases (Kanfi et al. 2010).

2.11 Glioma cell growth

The most common intracranial tumors in humans are gliomas (Armento et al., 2017). Malignant gliomas make up more than 80% of tumors that arise in the brain (Chen et al., 2014). Among all tumors, glioblastoma multiforme (GBM) is most malignant type tumor, with an incidence of 3-5 out of 100,000 people of the western world (Armento et al., 2017). Unfortunately, it is referred as one of the most familiar and most destructive malignant adult gliomas and has been associated with such an extremely low survival period (<15 months) (Stupp et al., 2005).

SIRT6 is involved in several intracellular processes that are similar to other sirtuins, for example, TNF-α secretion (Jiang et al., 2013) and lipid transport (Lee et al., 2014). It's indeed significant to observe that it helps to preserve genomic stability and gene repression. A tumor suppressor that controls the metabolism of cancer has recently been reported to be SIRT6, although this role of SIRT6 is still under doubt. However, the role of SIRT6 in gliomas is currently unclear.

In 2016, Feng et al. found that overexpression of SIRT6 in adenovirus prevented the growth of glioma cells across the two glioma cell lines (U87-MG and T98G) and provoked identified cell injury in glioma cells. The fluorescent terminal deoxyribonucleotidyl transferase (TDT) facilitated biotin-16-dUTP nick-end labeling (TUNEL) assay revealed that overexpression of SIRT6 accelerated apparent apoptosis in T98G glioma cells. Immunoblotting and immunofluorescent staining demonstrated how SIRT6 overexpression facilitated the mitochondrial-to-nuclear translocation with apoptosis-inducing factor (AIF) that is a potent apoptosis inducer. In addition, it is found that overexpression of SIRT6 decreased oxidative stress to a large extent and inhibited the regulation of the JAK2/STAT3 signaling pathway in glioma cells. Consequently, the levels of SIRT6 mRNA and protein have been shown to be considerably lower in multiforme tissues of human glioblastoma than in peritumour tissues. And also, SIRT6 suppresses the growth of glioma cells through apoptosis induction, oxidative stress inhibition and JAK2/STAT3 signaling pathway activation inhibition (Feng et al. 2016).

Afterwards, Chen et al. (2014) reports that the gene PCBP2 is over-expressed in human glioma tissues, however, the pathway by which PCBP2 has been regulated in glioma remains unknown. SIRT6 levels are down-regulated with the increase levels of PCBP2 in human glioma tissues. In the other observation, on the PCBP2 promoter, H3K9ac enhancement has been identified with the increased PCBP2 expression. So, SIRT6 can be targeted for PCBP2 expression. PCBP2 expression can be hindered by SIRT6 that is done with the deacetylating of H3K9ac. Then eventually, SIRT6 suppresses glioma cell growth and colony production in vitro and glioma cell proliferation in vivo in such a PCBP2-dependent manner (Chen et al., 2014). Such results indicate that SIRT6 can become potential therapeutic target for glioma treatment (Feng et al. 2016).

2.12 Cardiac hypertrophy

Cardiac hypertrophy is referred as the irregular expansion or thickening of the heart musc that arises from the changes in cardiomyocyte size and enhancements in other heart muscle components, for instance, the extracellular matrix (Nature Research). Since it is the adaptive response against, it gives pressure or volume stress, mutate sarcomeric (or other) protein, or loss the contractile mass from prior infarction. Hypertrophic development is accompanied by many other kinds of heart disease, and they are ischemic disease, hypertension, heart failure, and valvular disease. Perhaps by minimizing wall stress and oxygen intake, pressure overload-induced hypertrophy has assumed to provide a compensatory role in these types of cardiac pathology (Sandler & Dodge, 1963; Hood et al., 1968; Grossman et al., 1975).

For weakening human hearts and mouse hearts, SIRT6 expression is conducted to determine the role of SIRT6 within that progression of heart failure. After that, hypertrophy has been introduced whether through surgically developing transverse aortic constriction (TAC) or through incorporating hypertrophic agonists with isoproterenol (ISO) or angiotensin-II (Ang-II). Here, SIRT6 levels were significantly reduced in respect to control hearts in both human and mouse hearts, which suggests that SIRT6 deficiency is linked to the occurrence of cardiac hypertrophy and failure (Sundaresan et al. 2012).

Sundaresan et al. discovered in 2012 that sirtuin 6 (SIRT6) works at chromatin level to specifically attenuate insulin-like growth factor (IGF)-Akt signaling. In SIRT6 deficient mice, cardiac hypertrophy and heart failure emerged, while SIRT6 transgenic mice were protected from hypertrophic stimuli, clearly indicating that SIRT6 acts as a down regulation of cardiac hypertrophy. SIRT6-deficient mouse hearts showed hyperactivation and their expression levels of IGF signaling-related genes. Mechanistically, SIRT6 binds with the IGF signaling-related genes and suppresses their expression by communicating with c-Jun and deacetylating histone

3 at Lys9 (H3K9). These data suggest a new correlation among SIRT6 and IGF-Akt that indicate SIRT6 as a treatment option in cardiac hypertrophy (Sundaresan et al. 2012).

2.13 Pancreatic cancer

When uncontrolled cell growth starts in a portion of the pancreas, pancreatic cancer occurs. Tumors form, and these interfere with the functioning of the pancreas. Until the later phases, pancreatic cancer frequently displays no symptoms. It can be hard to handle for this reason. In the United States approximately 3 percent of all cancers are pancreatic cancers, according to the American Cancer Society (Brazier, 2018).

The relationship between carcinogenesis and inflammation has been established for many years (Coussens & Werb, 2002). And, chronic inflammation is a risk factor in the growth of cancer. In addition, in most of the cancers which do not develop in inflammatory cells, but an inflammatory component is often found and it is now understood to be an integral part of the malignant microenvironment (Hanahan & Weinberg, 2011; Balkwill & Mantovani, 2012). Inflammation progresses towards both tumorigenesis and cancer development through supplying growth factors that promote cancer cell proliferation and survival of them. Proangiogenic factors, the extracellular matrix-modifying enzymes that facilitate invading and metastasis and pass messages to direct epithelial to mesenchymal transition (Hanahan & Weinberg, 2011; Fernando et al., 2011; Mantovani, 2010). In addition, the systemic manifestations of disease are caused by elevated synthesis and secretion of pro-inflammatory cytokines, such as cachexia, fever and sweat (Kiefer & Siekmann, 2011; Argile's et al., 2009; Kurzrock, 2001; Robert et al., 2012). Simillarly, pancreatic ductal adenocarcinoma (PDAC) is better known for its tendency to secrete high levels of proinflammatory factors, among other forms of cancer, which then contribute to both its clinical aggression and metastatic spread (Hidalgo, 2010).

The secretion of cytokines by cancer cells makes a significant contribution to cancer-induced symptoms and angiogenesis. Due to the increased expression of TNF, studies have shown that SIRT6 promotes inflammation. Here, the purpose of Bauer et al. 2012 was to determine whether SIRT6 is involved in obtaining cancer cells with an inflammatory phenotype and to identify the mechanisms which link inflammation to SIRT6. Then, it has been shown that SIRT6 helps to enhance the expression of pro-inflammatory cyto-/chemokines, for example, IL8 and TNF, and facilitates cell migration in pancreatic cancer cells by boosting Ca2+ responses. Via its enzymatic action, SIRT6 helps to increase the intracellular levels of ADP-ribose that is a modulator of the Ca2+ and TRPM2 receptor. So, TRPM2 and Ca2+ are found to be involved in SIRT6-induced TNF and IL8 expression. SIRT6 raises the transcription factor that is dependent on Ca2+, activated nuclear T cell factor (NFAT) and cyclosporin A. These results indicate some feature of SIRT6 for promoting pancreatic cancer, which are in the synthesis of Ca2+-mobilizing second messengers, in the regulation of Ca2+-dependent transcription factors, and in the expression of cytokines that are pro-inflammatory, proangiogenic and chemotactic. Suppression of SIRT6 can assist to combat cancer-induced inflammation, angiogenesis and metastasis (Bauer et al. 2012).

2.14 Type 2 diabetes

According to the World Health Organization (WHO), type 2 diabetes mellitus (T2DM) is an emerging disease that has a correlation with obesity and affects hundreds of millions of people worldwide, accounting for about 10% of the world's population. Sedentary lifestyles and high calorie intake are the main contributors to the rapid rises of both T2DM and obesity. In curing obesity-associated diseases, changes in behaviors such as exercise and diet are significant, yet their effect from a practical point of view is minimal and limited. In market, here are a variety of anti-diabetic medications, among those metformin is being used as a first-line therapy. But

these treatments do not yield satisfactory results. Because of the adverse effects of existing drugs and long-lasting and painful T2DM morbidities, novel therapies are desperately needed.

In 2006, Mostoslavsky et al. observed an extreme hypoglycemia in SIRT6-null mice linked to increased liver and uptake of skeletal glucose and insulin signaling revealed the main role of SIRT6 in homeostasis of glucose. Besides, Kanfi et al. (2008) found that the expression of SIRT6 proteins is regulated by nutrient availability. Deficiency of nutrients (glucose and serum) in cell cultures and for fasting animals, SIRT6 levels are elevated. The levels of SIRT6 throughout the heart, kidney, brain and white adipose tissue are enhanced in mice after calorie reduction (Kanfi et al. 2008). On the other hand, SIRT6 levels become down-regulated in animals and humans that are insulin-resistant (de Kreutzenberg et al. 2010; Kim et al. 2010), demonstrating that it is necessary to maintain the level or activity of SIRT6 to avoid metabolic diseases.

One research has documented that rosiglitazone, an agonist of peroxisome proliferator activated receptor c (PPARc), regulates the expression and function of SIRT6 genes in rat liver tissue (Yang et al. 2014). The SIRT6 knockdown abolished rosiglitazone's fatty liver disease (FLD)-suppressing effects, suggesting that SIRT6 is a significant regulator of the metabolic processes influenced by rosiglitazone. In addition, Kanfi et al. 2010 reports that SIRT6 treated transgenic mice given high-fat diet (HFD) secreted more insulin than their wild counterparts in response to glucose loading. This outcome indicated that β-cell function and insulin secretion may have involvement with SIRT6. Futhermore, visceral fat accumulation, LDL-cholesterol, and TGs were drastically decreased while the SIRT6 function in metabolic stress was investigated in SIRT6 over-expressing transgenic mice (Kanfi et al. 2010). Interestingly, Anderson et al. 2015 found that when SIRT6 is over-expressed in mice, the insulin-stimulated glucose deposition in skeletal muscle was enhanced despite the changes in body weight or adiposity. These roles identified by genetic studies involve fostering pancreatic insulin

secretion, inhibiting hepatic gluconeogenesis and triglyceride production, and suppressing adiposity, indicating that SIRT6 activators are useful molecules for the treatment of obesity and diabetes.

In contrast, a recent study by Bae (2017) showed that a synthetic SIRT6 inhibitor improved the tolerance to glucose in the type 2 diabetes mouse model, along with increased glycolysis and expression of the skeletal muscle GLUT-1 and 4 glucose transporter which give evidence of SIRT6 inhibition as a treatment for diabetes. These contradictory effects of SIRT6 on metabolism indicate that there may be therapeutic potential for both activation and inhibition of SIRT6 deacetylase activity against T2DM (Bae, 2017).

Chapter 4: Conclusion and Future Directions

This review inspected all the available role of SIRT6 in different diseases that are caused by pathogenesis of smooth muscle and epithelial tissue. Over the years, SIRT6 has been studied and many protective roles to treat various diseases has found. Likewise, its advantageous effects are researched and determined which can provide a useful therapeutic target for those diseases.

Although many investigations have been performed about SIRT6 protein, it lacks sufficient data to understand clearly of its effectiveness and risk factors. Hence, more clinical trials and studies can be carried out for evaluating treatment strategies with SIRT6 in future.

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