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The Protective Role of Endothelial SIRT1 Against Vascular Aging and Hypertension

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Abstract

Hypertension is a major risk factor for cardiovascular diseases and increases with age. Vascular aging also predisposes older individuals to hypertension. SIRT1 has emerged as an important suppressor of aging-related changes in the vascular endothelium. This study investigated the protective effects of endothelial SIRT1 against vascular aging and hypertension using transgenic mouse models with altered SIRT1 expression. Three mouse models were studied: wild-type (WT) mice, endothelial cell-specific SIRT1 overexpressing (EC-SIRT1) mice, and endothelial cell-specific dominant negative SIRT1 overexpressing (EC-H363Y) mice. Body weight, blood glucose levels, blood pressure, vascular function, senescence staining, and eNOS expression were analyzed. Results demonstrated that endothelial SIRT1 overexpression protects against vascular aging and hypertension, while dysfunctional SIRT1 promotes these effects. This provides insight into SIRT1's role in delaying vascular aging and its potential as a therapeutic target.

Introduction

Hypertension is one of the strongest risk factor leading to cardiovascular diseases, and this risk increases with aging[1]. As people age, body cells lose the ability to divide, and are known as senescence cells[2]. Cellular senescence paired with hardening of blood vessels leads to vascular aging[3,4]. Vascular aging predisposes older individuals to hypertension and other cardiovascular conditions[4]. It is therefore important to understand the mechanisms underlying vascular aging and hypertension and how it can be delayed or prevented.

The inner lining of blood vessel, called the endothelium, is majorly involved in the control of the vascular tone, which is the contraction and relaxation of blood vessels to allow blood flow[5]. Chronic stressors like aging, obesity, diabetes, etc. can impair normal endothelial function, causing impaired vascular tone and promotes vascular disease[3,4]. Sirtuin 1 (SIRT1), belonging to a large family of sirtuins, is a gene that regulates aging and longevity at multiple sites in the body, including on the vascular endothelium[4]. Endothelial SIRT1 has emerged as an important suppressor of aging-related changes in the vascular endothelium[4].

The expression and activity of endothelial SIRT1 is shown to decline with age[6]. When SIRT1 is overexpressed, it helps prevent of endothelial cells senescence and vascular stiffening[4]. For instance, SIRT1 regulates endothelial function by activating endothelial Nitric Oxide Synthase (eNOS), an enzyme that promotes dilation of blood vessels through production of a gaseous substance known as nitric oxide (NO)[4].

In this study, the beneficial effects of SIRT1 overexpression in the vascular endothelium against vascular aging and hypertension were investigated using transgenic mice models with altered endothelial SIRT1 expressions. The research results could provide insight into the various mechanisms by which SIRT1 delays vascular aging and identify it as a potential therapeutic target for age-related vascular diseases.

Methodology

To understand the different ways that endothelial SIRT1 protects against vascular aging and hypertension, three types of mice model were studied; normal mice without any genetic modification, known as wild-type (WT) mice, mice with overexpression of human vascular endothelial SIRT1, known as EC-SIRT1 mice, and mice with overexpression of a mutant and non-functional SIRT1 in the vascular endothelium, known as EC-H363Y mice. The mice were at around 20 to 30 weeks old throughout the experiments.

Six experiments were carried out to achieve the objective of this study:

Body weight of mice were measured to observe their weight change pattern. This was done by weighting mice on balance. Mice weight were measured weekly for 4 weeks.

Circulating blood glucose of mice were studied. Mice were fasted overnight, and their initial blood glucose level was measured using a glucometer. Glucose was administered to mice intraperitoneally, and their blood glucose level were measured from 5 to 120-minute time points. This allowed observation of mice's ability to metabolize glucose.

Systolic blood pressure of mice was measured using the tail-cuff Blood Pressure Analysis System.

Vascular function analysis was performed with wire myography system to study the contraction and relaxation of the blood vessel called aorta that was surgically removed from the mice at the end of the experiment.

Senescence staining of full-length aorta was performed using SA-β-galactosidase activity marker to observe the number of senescent cells in the endothelial lining of mice aorta.

Protein expression of eNOS was measured in mice aorta using Western Blotting.

Results

The results of each experiment is shown below.

Body Weight and Circulating Blood Glucose:

Body weight of mice of different genotype were measured over 4 weeks, and the percentage change in their body weight from week 1 and week 4 was calculated. From figure 1, the percentage change in body weight of EC-H363Y mice can be seen to be the highest, having around 8% increase in body weight over 4 weeks. WT mice have the second highest percentage change in body weight at around 4%. While EC-SIRT1 mice have the lowest change, with increase of only 2% in 4 weeks.

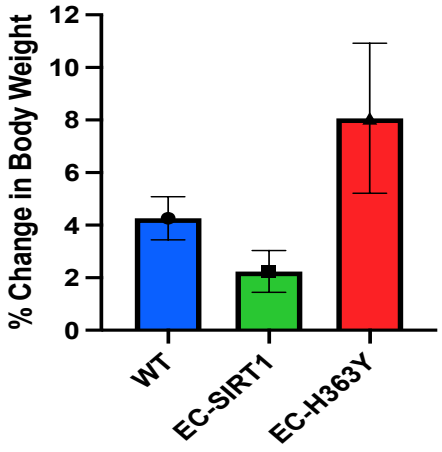

Figure 1:Percentage change in body weight of WT, EC-SIRT1 and EC-H363Y mice. Percentage change of body weight in EC-H363Y mice is the highest, second highest is WT mice, and lowest is EC-SIRT1.

Glucose was administered to fasting mice and their blood glucose levels were measured before and after glucose administration at different time points. The change in the blood glucose level was calculated. Referring to figure 2, the blood glucose level of EC-SIRT1 mice increased significantly after glucose administration and showed the highest increase among the 3 genotypes. On the contrary, the blood glucose level of EC-H363Y mice increase the least, having almost half the increase seen in EC-SIRT1 mice. The blood glucose level of the WT mice was less than EC-SIRT1 mice but more than the EC-H363Y mice.

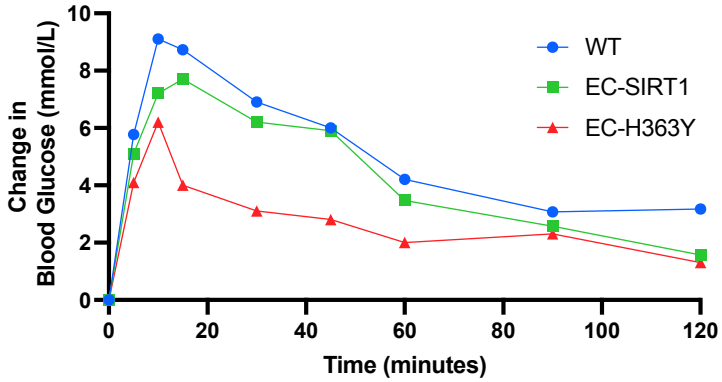

Figure 2: Change in circulating blood glucose level of WT, EC-SIRT1 and EC-H363Y mice. EC-SIRT1 mice had the highest change in blood glucose level after administrating glucose to fasting mice, WT mice had the second highest change, and EC-H363Y mice had the lowest change.

Systolic Blood Pressure and Vascular Function Analysis:

The systolic blood pressure of the mice was measured. From figure 3, EC-H363Y mice had significantly higher blood pressure than WT and EC-SIRT1 mice, reaching 130 mmHg. WT and EC-SIRT1 have lower blood pressure, at the healthy point of around 100- 110 mmHg, though EC-SIRT1 mice has the lowest blood pressure among the three genotypes. The change in tension of mice aorta in wire myography system was measured to analyze vascular function of aortae of different mice genotypes. From results shown in figure 4, EC-H363Y mice had

the highest tension, WT mice had the second highest while EC-SIRT1 mice had the lowest tension in aorta when contracting.

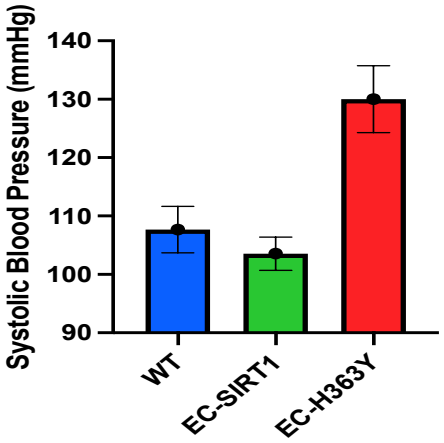

Figure 3: Systolic blood pressure of WT, EC-SIRT1 and EC-H363Y mice. EC-H3636Y mice have the highest blood pressure, while EC-SIRT1 mice have the lowest. WT mice have slightly higher blood pressure than EC-SIRT1 mice.

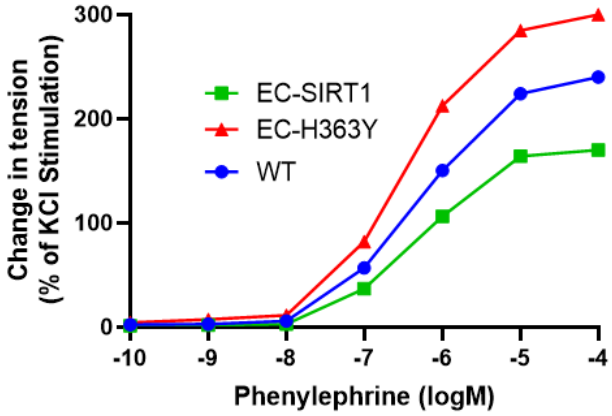

Figure 4: Vascular function analysis showing change in tension of WT, EC-SIRT1 and EC-H363Y mice aortae. EC-H363Y 1 mice show the highest tension, WT mice second highest and EC-SIRT1 have the lowest tension.

Senescence Staining and eNOS expression:

To observe cellular senescence, senesce staining of full-length aortae was performed. The blue stains indicate senescent cells. From figure 5, it can be observed that EC-H363Y mice

have high number of senescent cells, as competed to WT and EC-SIRT1 mice which show almost no signs of senescence.

*Figure 5: Senescent staining of full-length aortae of WT (*Veterian Key 2016*), EC-SIRT1 and EC-H363Y mice. EC-H363Y mice indicate high number of senescent cells, as indicated by blue stains.*

Expression of eNOS in the aortae of mice was measured using western plotting method. In figure 6, EC-SIRT1 mice have the highest expression of eNOS, WT mice also show eNOS expression though it's significantly less. In EC-H363Y mice, there is almost no expression of eNOS. The eNOS expression is compared to Beta-Actin, this is used as a control as Beta-Actin is a protein present in all cells.

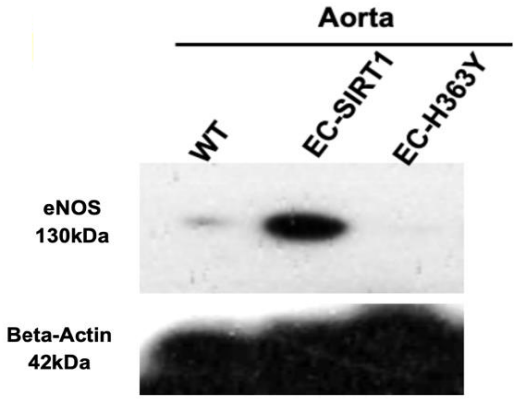

Figure 6: Protein expression of eNOS measured on mice aortae using western blotting. The expression of eNOS is highest in EC-SIRT1 mice, eNOS is expressed slightly in WT and almost show no expression in EC-H363Y mice aorta.

Discussion

The results provide important insights into the protective mechanisms of endothelial SIRT1 against vascular aging and hypertension.

The body weight and blood glucose data indicate SIRT1 plays a key role in metabolic regulation. SIRT1 is known to modulate the expression of genes involved in metabolism[7]. The overexpression of functional SIRT1 in EC-SIRT1 improved glucose homeostasis and prevented weight gain. In

contrast, the dysfunctional SIRT1 in EC-H363Y mice appeared to impair metabolism, leading to inefficient glucose handling and greater weight accumulation over time. Overall, results from the body weight measurement and circulating blood glucose level analysis provided insights into the importance of functional SIRT1 for effective metabolism.

The higher blood pressure observed in EC-H363Y mice (figure 3) suggests dysfunctional SIRT1 promoted increased vascular contraction as a phenotype of vascular dysfunction. A key finding was the increased contraction of EC-H363Y aortae seen on myography (figure 4) resulting from lack of functional SIRT1. This also implies that the increased blood pressure shown in figure 3 is due to excessive contraction of blood vessels as a result of SIRT1 dysfunction. The vascular function data and blood pressure data show that absence of functional SIRT1 can lead to increased vascular contraction and higher blood pressure and can cause hypertension.

SIRT1 enhances eNOS-mediated Nitric Oxide (NO) production[4]. NO regulates blood pressure and promotes vessel dilation[4]. The reduced eNOS levels in EC-H363Y mice as seen in figure 6 helps explain the elevated blood pressure and vessel contraction of EC-H363Y mice due to reduce NO bioavailability.

A notable result was the substantial increase in cellular senescence detected within the aged EC-H363Y aortas. This supports the concept that SIRT1 acts as an important suppressor of vascular aging by preventing senescence[4]. Cellular senescence has been linked to pathological vascular remodelling and promotion of age-related diseases such as hypertension[4,8]. The senescent cells present in EC-H363Y vessels likely further intensified vascular dysfunction and blood pressure through senescence-associated secretory phenotypes, such as pro-inflammatory signals[4,8]. Furthermore, hypertension is known to increase cellular senescence[9]. From data in figure 3, EC-H363Y mice show sign of hypertension due to overly high blood pressure, this gives potential explanation to the high number of senescent cells in aorta of EC-H363Y mice

Overall, the findings provide compelling evidence that endothelial SIRT1 protects the vasculature through modulating metabolism, eNOS production, and senescence prevention, establishing SIRT1 as an anti-aging target. Maximizing SIRT1 activity through gene therapy or pharmacological agents deserves investigation to counteract vascular aging and age-related conditions in older patients. This could have major clinical impacts on cardiovascular health in aging populations worldwide.

Conclusion

This study demonstrated that overexpression of SIRT1 in the vascular endothelium is protective against vascular aging and hypertension. Dysfunctional negative dominant SIRT1 expression in the endothelium promoted vascular aging and increased blood pressure. Further research is should be carried out to investigate the therapeutic potential of SIRT1, to utilize this gene as an important tool in the fight against an aging population globally and its attending cardiovascular disease burden.

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