**Summary**

A quick PubMed search for articles containing the keyword “Sirtuins” generates more than 4,000 papers, and the number has rapidly increased in recent years. The role of sirtuin in biochemistry, physiology and clinical medicine has been noticed as a key factor for metabolic and age-related diseases. However, there is little information about sirtuins in veterinary medicine. On the other hand, increasing in metabolic and age-related diseases is also a major problem in veterinary medicine in recent years, and development of the early diagnosis and prevention method for the above diseases is urgent subject for veterinary medicine. The aim of this study was to reveal the molecular mechanisms of sirtuins in inflammation of cat tissues.

1. Mammalian sirtuins have been identified as homologs of the yeast silent information regulator 2 (Sir2). Mammalian sirtuins included seven family and each has different target proteins. Most tellingly, SIRT1 and SIRT3 are induced by calorie restriction (CR), and related to the metabolic and age-related diseases in human and mouse. However, very little information is available on cat SIRT1 and SIRT3. Therefore, we considered that to obtain the basic knowledge about cats SIRT1 and SIRT3 is necessary. We determined the cat SIRT1 and SIRT3 cDNA sequences and examined their mRNA expression in several tissues. We successfully cloned the cat SIRT1 and SIRT3 cDNAs. Cat SIRT1 and SIRT3 showed high sequence homology with other vertebrate SIRT1 (>61.3%) and SIRT3 (>65.9%), respectively. Cat SIRT1 and SIRT3 were highly conserved, and they showed especially high homology in the catalytic core domain. This core domain included in zinc fingers and $NAD^+$ binding sites. SIRT1 and SIRT3 were genetically conserved in the phylogenetic tree, and may have functions similar to those of other animals. The results of real time PCR

using tissue total RNA revealed that cat SIRT1 and SIRT3 mRNA were expressed in various tissues similar to other animals. High expression were observed in the liver and skeletal muscle for SIRT1 and in the heart for SIRT3. From the above results, cat SIRT1 and SIRT3 are expected to have various physiological activities as well as other animals. Further examinations of thier detail function and relationship with diseases are necessary.

2. Recently, prevalence of obesity has increased also in cats. Lipotoxicity observed in obese animals seems to be fundamental pathogenesis for various metabolic diseases including diabetes mellitus. SIRT1 and SIRT3 have been considered to play important roles in molecular mechanism of obesity onset particularly via inflammation. However, very little information is available on mechanism of lipotoxicity and the role of sirtuins in cats. Therefore, we induced obesity by feeding on high-fat diet (HFD) for 8 weeks in cats, and investigated expressions of inflammatory makers, cytokines, SIRT1 and SIRT3 in peripheral leukocytes. Body weights of cats significantly increased, but other metabolic markers did not change after HFD feeding. Hepatic injury markers, ALT, ALP and AST activities, significantly increased by HFD feeding. Although peripheral leukocyte inflammatory cytokine mRNA expression did not increase, mRNA expression of SIRT1 significantly increased by HFD. From the above results, we consider that inflammation is induced by lipotoxicity in the liver, and inflammatory signals are suppressed by SIRT1 in the peripheral leukocyte after HFD feeding. We consider that SIRT1 is an important molecules to suppress the inflammation concerning the onset of metabolic and age-related diseases.

3. Regulation of nuclear factor kappa B (NF-κB) is central role in the anti-inflammatory function by SIRT1. Post translational modification of p65

subunit of NF-κB (p65) is the main route of regulation of NF-κB transcriptional activity by SIRT1. NF-κB contributes to various diseases including metabolic and age-related diseases through chronic inflammation by activating pro-inflammatory cytokine production. NF-κB seems to relate to onset of various diseases, and clinical pathological research targeting cat p65 has been reported. However, very little information is available on the molecular characterization of cat p65. To obtain the basic knowledge of cat p65, we cloned and characterized cat p65, and examined their immune regulatory function. We successfully cloned the cat p65. The deduced amino acid sequence was highly conserved in mammal p65 (>87.5%). In particular, functional domains were conserved very well. The amino acid residues, which undergo post-translational modifications in mammals, were completely conserved in cat p65. The cat p65 mRNAs were expressed in all examined tissues as reported in other animals. In particular, high expression levels of cat p65 were observed in adipose tissue, heart and skeletal muscle. Transiently expressed cat p65 significantly up-regulated NF-κB transcriptional activity and pro-inflammatory cytokine expression in cat fibroblast tissues. Therefore, cat p65 may have important roles in inflammation and SIRT1 may be involved in the regulation of inflammation.

4. Relationship between SIRT1 and NF-κB in chronic inflammation has important effects on onset of metabolic and age-related diseases in animals. Chronic inflammation is occurred by persistent low level of physiological inflammation through the response to the endogenous-exogenous stress. Sustained chronic inflammation causes organ dysfunction by failure of adaptation. Fibroblasts are involved in wound healing by synthesizing of extracellular matrix in tissues. In addition, fibroblasts produce inflammatory cytokines and modify the level of inflammation. For these reasons, fibroblasts

are considered an important factor in the formation of chronic inflammation. SIRT1 activity of regulating inflammation through NF-κB in fibroblast is important in various diseases. However, there is little information in cats. Therefore, we analyzed the effect of NF-κB transcriptional activity and inflammatory cytokine production by SIRT1 in cat fibroblast cells. Transiently expression of SIRT1 suppressed the NF-κB transcriptional activity and pro-inflammatory cytokine expression by cat p65 and LPS in cat fibroblast. These result revealed that SIRT1 inhibit the NF-κB signals and suppress the inflammation in cat fibroblast cells. We consider that SIRT1 is concerned in onset of metabolic and age-related diseases through suppression of chronic inflammation.

In conclusion, cat SIRT1 has anti-inflammatory function via NF-κB. Chronic inflammation causes lipotoxicity and subsequently onset of metabolic and age-related diseases. We consider that SIRT1 involving in the occurrence of chronic inflammation relate to onset of metabolic and age-related diseases. Further study is needed to elucidate the detail molecular mechanisms. Sirtuins are applied to early diagnosis and prevention as a biomarker of various diseases in human. Molecules having high activating ability of sirtuins are detected and it has been applied to drug development. We expect that this study contributes a little to the clinical applications of sirtuins in cats.