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# Expression of Sirt1, Sirt2, Sirt5, and Sirt6 in the Mouse Testis

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## ABSTRACT

**Sirtuin proteins are evolutionary conserved Sir2-related NAD+-dependent deacetylases and regulate many of cellular processes such as metabolism, inflammation, transcription, and aging. Sirtuin contains activity of either ADP-ribosyltransferase or deacetyltranfease and their activity is dependent on the localization in cells. However, the expression pattern of Sirtuins has not been well studied. To examine the expression levels of Sirtuins, RT-PCR was performed using total RNAs from various tissues including liver, small intestine, heart, brain, kidney, lung, spleen, stomach, uterus, ovary, and testis. Sirtuins were highly expressed in most of tissues including the testis. Immunostaining assay showed that Sirt1 and Sirt6 were mainly located in the nucleus of germ cells, spermatocytes, and spermatids in the seminiferous tubules, whereas Sirt2 and Sirt5 were exclusively present in the cytoplasm of germ cells and spermatocytes. Our results indicate that Sirtuins may function as regulators of spermatogenesis and their activities might be dependent on their location in the seminiferous tubules.**

**(Key words : Sirtuin, Sirt1, Sirt2, Sirt5, Sirt6, Mouse, Testis)**

## INTRODUCTION

Sirtuin proteins are a class of Sir2-related NAD+-dependent deacetylases (North and Verdin, 2004). Sirtuin contains 7 family members including Sirt1, Sirt2, Sirt3, Sirt4, Sirt5, Sirt6, and Sirt7. Sirtuins possess two enzyme activities for mono-ADP-ribosyltransferase and deacetyltranfease. Sirtuins are involved in numerous cellular processes including ageing, transcriptional regulation, apoptosis, inflammatory response, stress resistance, and energy balancing (Galli *et al.*, 2011; North and Verdin, 2004; Preyat and Leo, 2013; Shoba *et al.*, 2009; Taylor *et al.*, 2008).

The functions of Sirtuin proteins were multiple and depending on their location in the cells as they are various. Sirt1 localizes to both the nucleus and the cytosol of cells, whereas Sirt2 is mainly located at the cytosol (Afshar and Murnane, 1999; Perrod *et al.*, 2001). Sirt3, Sirt4, and Sirt5 are present in the mitochondrial compartment (Nakamura *et al.*, 2008). Sirt6 is a nuclear ADP-ribosyltransferase (Liszt *et al.*, 2005). Sirt7 is predominantly limited in the nucleus (Kiran *et al.*, 2013).

The present study was performed to look into the location of Sirtuins such as Sirt1, Sirt2, Sirt5, and Sirt6 in the mouse testis.

## MATERIALS AND METHODS

### Animals

All mice experiments were performed on 6~10 week-old ICR mice provided by Orient Bio Company (Seongnam, Korea). Mice were housed under temperature and light controlled conditions with the lights on for 12 hours daily and given with a free access to food and water. Care of mice and experimental procedures were conducted with the Guide for the Care and Use of Laboratory Animals. All experiments were approved by the Institutional Animal Care and Use Committee of CHA University. Mouse testis was frozen and stored at −80℃ to isolate RNA and protein extraction, or fixed in 4% PFA solution to make paraffin block.

### RT-PCR Analysis

Total RNA from testis was isolated using Trizol, as per the manufacturers' instruction (Invitrogen, Carlsbad, CA, USA). Two microgram of total RNA was converted to cDNA using TOP script™ cDNA Synthesis kit (Enzynomics, Daejeon, Korea), followed by PCR amplification. The primers used for RT-PCR were shown in Table 1.

### Immunostaining

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**Table 1. Oligonucleotide sequences for RT-PCR analysis**

| Gene ID | | 5' → 3' | Size |
|---|---|---|---|
| *Sirt1* | F | ATGACGCTGTGGCAGATTGTT | 202 bp |
| | R | CCGCAAGGCGAGCATAGAT | |
| *Sirt2* | F | GCCTGGGTTCCCAAAAGGAG | 145 bp |
| | R | GAGCGGAAGTCAGGGATACC | |
| *Sirt3* | F | ATCCCGGACTTCAGATCCCC | 126 bp |
| | R | CAACATGAAAAAGGGCTTGGG | |
| *Sirt4* | F | GGCGACGTGTTCCTCACTG | 131 bp |
| | R | ACAAAGTCAACCTTGTCTGGG | |
| *Sirt5* | F | CTCCGGGCCGATTCATTTCC | 130 bp |
| | R | GCGTTCGCAAAACACTTCCG | |
| *Sirt6* | F | ATGTCGGTGAATTATGCAGCA | 138 bp |
| | R | GCTGGAGGACTGCCACATTA | |
| *Sirt7* | F | CAGGTGTCACGCATCCTGAG | 231 bp |
| | R | GCCCGTGTAGACAACCAAGT | |

The deparaffinized tissue section was incubated with blocking buffer (4% bovine serum albumin and 5% normal IgG in PBS) for 1hour at room temperature and incubated with primary antibody in the blocking buffer for overnight at 4℃. The antibodies used in this study were anti-Sirt1 (1:200, Abcam), Sirt2 (1:200, Abcam), Sirt5 (1:200, Abcam), and Sirt6 (1:500, Millipore) antibodies. After washing with PBS, the sections were incubated with the secondary antibodies (1:1,000) in blocking buffer for 2 hours at room temperature. The testis sections were washed with PBS and mounted. DAPI was used for nuclear counter stating of testis section. Slides were viewed and imaged with a confocal microscope, LSM 510 Meta (Carl Zeiss Co., Germany) equipped with three lasers.

## RESULTS

To examine the expression of *Sirtuin* family members in tissues, RT-PCR analysis was performed using total RNAs from various tissues; small intestine, stomach, kidney, liver, heart, brain, lung, uterus, ovary, and testis (Fig. 1). The expression patterns of the 7 *Sirtuin* genes are shown in Fig. 1. *Sirt2, Sirt3, Sirt4* and *Sirt5* were expressed in most of tissues tested. On the other hand, the expression of *Sirt1, Sirt6* and *Sirt7* were likely to be limited in several tissues including the testis and the ovary (Fig. 1).

To investigate the localization of Sirtuin family members in mouse testis, immunofluorescence and immunocytochemistry were performed using antibodies against mouse Sirt1, Sirt2, Sirt5 and Sirt6 with the stage specific markers, either Sycp3 or Ddx4. Sirt1 was highly detected in the seminiferous tubules of the mouse testis (Fig. 2). Sirt1 mainly located at spermatocyte which was co-localized with Sycp3 which is one of markers for spermatocytes in the testis (de la Fuente *et al.*, 2007). Sirt6 was detected in the seminiferous tubules of the mouse testis (Fig. 2). Sirt6 was also located in most

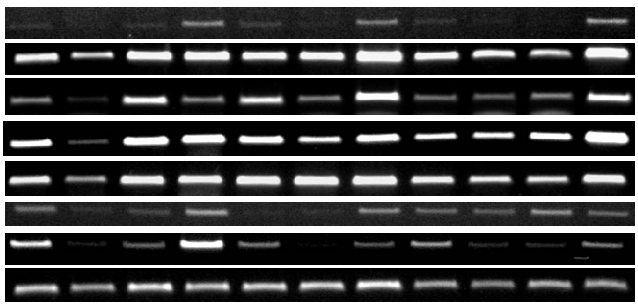

**Fig. 1. Gene expression of Sirtuin family members in mouse tissues**. Expression of Sirtuin family members, Sirt1, Sirt2, Sirt3, Sirt4, Sirt5, Sirt6, and Sirt7, were analyzed by RT-PCR. Total RNAs were extracted from mouse tissues; small intestine, stomach, kidney, spleen, liver, heart, brain, lung, uterus, ovary and testis. *Gapdh* was used as control. Primers were shown in Table 1.

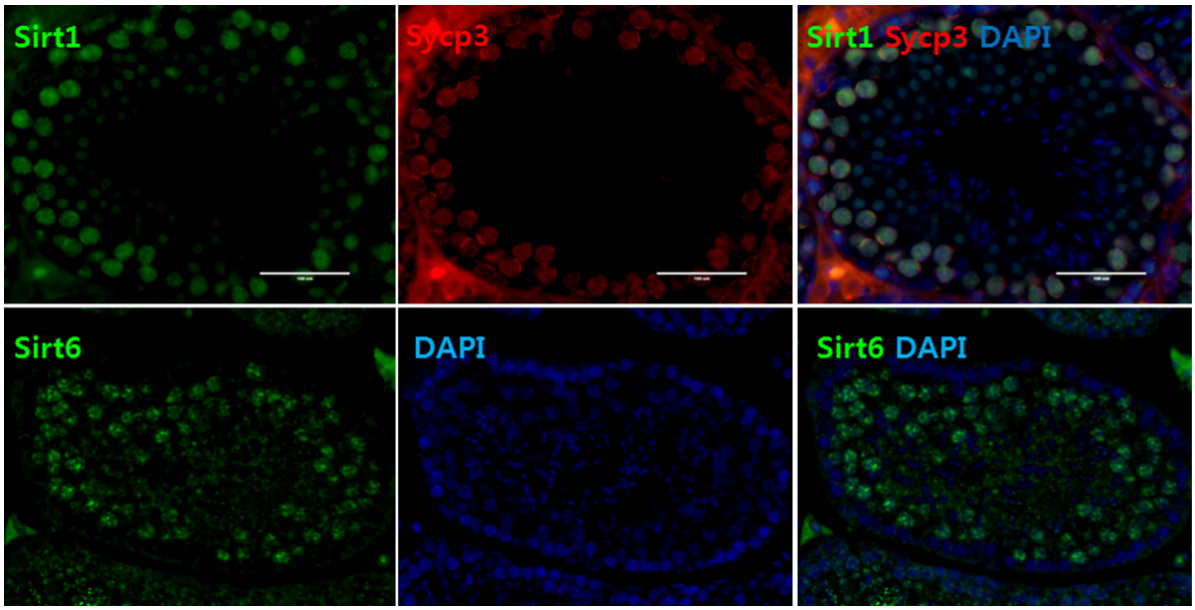


**Fig. 2. Localization of Sirt1 and Sirt6 in the seminiferous tubule in the testis.** Co-immunostaining of testis with antibodies against Sycp3 and Sirt1 or Sirt6 using immunofluorescence. Tissue sections were prepared from 6~10 weeks-old testis. White scale bars indicate 100 μm.

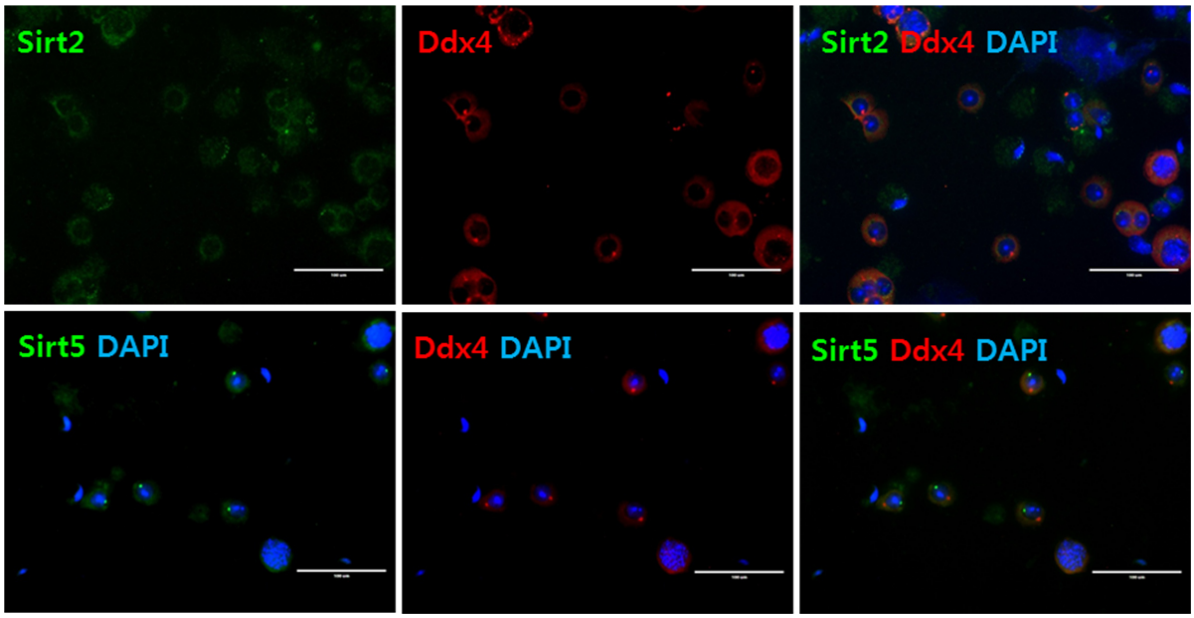


**Fig. 3. Localization of Sirt2 and Sirt5 in the seminiferous tubule in the testis.** Co-immunostaining of testis with antibodies against Ddx4 and Sirt2 or Sirt5 using immunohistochemistry. Tissue sections were prepared from 6~10 weeks-old testis. White scale bars indicate 100 μm.

of cell types including spermatogonia, spermatocyte and round spermatid.

The expression and location of Sirt2 and Sirt5 was look into by immunocytochemistry using cells separated from mouse testis (Fig. 3). Sirt2 was located in the cytoplasm in the germ cells and dividing spermatocytes. Sirt2 was co-localized with Ddx4 which is known as Vasa, germ cell marker (Noce *et al.*, 2001). Sirt5 was also detected in the cytoplasm in the germ cells (Fig. 3). Sirt5 was co-localized with Ddx4 in the

germ cells.

## DISCUSSION

The present study showed that Sirtuins such as *Sirt1, Sirt2, Sirt3, Sirt4, Sirt5, Sirt6* and *Sirt7* expressed in the mouse testis. To localize their expression in the testis, we performed immunostaining using each specific antibody. However, only Sirt1, Sirt2, Sirt5 and Sirt6 were detected by immunofluorescence and immunocytochemistry. Sirt1 and Sirt6 located in the nucleus of spermatogonia and spermatocyte during spermatogenesis in the seminiferous tubule, whereas Sirt2 and Sirt5 were present in the cytoplasm of the cells.

Recent studies reported that Sirtuins were localized in different subcellular compartments such as the nucleus, the cytosol, the mitochondria, the nucleolus in cells. Sirt1 possesses two nuclear localization signals (NLS) and two exportation signals (NES) (Tanno *et al.*, 2007). Therefore, Sirt1 is able to locate in either the nuclear or the cytoplasmic compartment depending on the cell type or tissue. In COS-7 cells, Sirt1 is mainly present in the nuclear compartment (McBurney *et al.*, 2003; Sakamoto *et al.*, 2004), whereas it is localized in the cytoplasm of β cells, myotubes, and cardiomyocytes (Moynihan *et al.*, 2005; Tanno *et al.*, 2007). Our results showed that Sirt1 in the testis were predominantly present in the nucleus of the germ cells at seminiferous tubules. Sirt1 is involved in chromatin remodeling by regulating deacetylation of histones (Vaquero *et al.*, 2004). The epigenetic regulation during spermatogenesis is very important. Disruption of Sirt1 resulted in attenuation of spermatogenesis (Coussens *et al.*, 2008). Recent report suggested that Sirt1 catalytic activity is necessary for male fertility (Seifert *et al.*, 2012). Sirt6 contains a nuclear localization signal (Flick and Luscher, 2012). Therefore, Sirt6 is predominantly present in the nucleus like Sirt1 (Liszt *et al.*, 2005). Loss of Sirt6 showed degeneration of multiple organs by genomic instability and aging resulting in early death (Mostoslavsky *et al.*, 2006). Sirt6 might be involved in DNA repair during spermatogenesis in the testis. Sirt2 possesses a nuclear exportation signal, therefore it is only present in the cytoplasm (North and Verdin, 2004; Wilson *et al.*, 2006). Sirt2 is involved in microtubule network to control cell cycles (North *et al.*, 2003). Male germ cells undergo dynamic meiotic process during spermatogenesis in the testis. In the cytoplasm of spermatogonia and spermatocyte, Sirt2 might orchestrate meiotic process via control of microtubule network. Sirt5 is involved in regulation of urea cycle via regulation of a mitochondrial enzyme, carbamoyl phosphate synthetase 1 (CPS1) (Michishita *et al.*, 2005; Nakagawa *et al.*, 2009). The control of CPS1 activity by Sirt5 might be important for detoxification in the cells. Round spermatid undergoes spermiogenesis to make it sperm with mitochondria assembly at tail of sperms. It implies that Sirt5 is able to play a role in regulation of mitochondria and physiological stress during spermatogenesis.

In conclusion, our results showed that Sirtuin members, such as Sirt1, Sirt2, Sirt5 and Sirt6 express in either the nucleus or the cytosol of spermatogonia, spermatocyte, and round spermatid. They might act as a regulator to control several cellular processes in the nucleus, cytosol and mitochondria during spermatogenesis in the testis.

## ACKNOWLEDGMENTS

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