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Review article

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SIRT7 AN EMERGING SIRTUIN: DECIPHERING NEWER ROLES

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Sirtuin7 (SIRT7) is a NAD^+-dependent protein deacetylase which belongs to sirtuin family. Although sirtuin proteins have attracted a great deal of attention, little is known about SIRT7. SIRT7 participates in rDNA transcription in the nucleolus. However, recent reports show that SIRT7 has additional novel functions. This review summarizes all findings about SIRT7, and highlights its critical roles in cellular functions.

Key words: *sirutins, SIRT7, oncogene, rDNA transcription, proliferation, ageing*

INTRODUCTION

The mammalian sirtuin family comprises seven proteins, designated as sirtuin1-7. Sirtuins possess core domains that catalyze their enzymatic activities, including nicotinamide adenine dinucleotide (NAD^+)-dependent-deacetylase, mono-ADP-ribosyltransferase, and possibly deacylase functions (1). Based on phylogenetic analysis, mammalian sirtuins are divided into four classes: Class I, including sirtuin1 (SIRT1), sirtuin2 (SIRT2), and sirtuin3 (SIRT3); Class II, sirtuin4 (SIRT4); Class III, sirtuin5 (SIRT5); and Class IV, sirtuin6 (SIRT6) and sirtuin7 (SIRT7) (2, 3). The sirtuins are also distinguished by their subcellular localizations. SIRT1, SIRT6, and SIRT7 are localized to the nucleus, whereas SIRT2-SIRT5 are localized to non-nuclear regions, with SIRT2 in the cytosol and SIRT3-SIRT5 in mitochondria (4). The diverse cellular functions mediated by sirtuins are involved in a wide range of physiological processes including the cell cycle, proliferation, apoptosis, senescence, genomic stability, and metabolism (5). Therefore, dysregulation of sirtuins is linked to several pathological conditions such as cancer, neurodegenerative disease, and metabolic disease (5, 6). Although the other sirtuins have been extensively studied by many groups, SIRT7 has received comparatively less attention. This review summarizes the current knowledge concerning functions and regulation of SIRT7.

SUBCELLULAR LOCALIZATION OF SIRT7

SIRT7 gene is located on chromosome 17q25.3, a region that is frequently altered in acute leukemias and lymphomas (7). The SIRT7 protein contains 400 amino acids, with 39% and 21% similarity to human SIRT1 and yeast Sir2, respectively (7). SIRT7 is localized to the nucleus. In interphase, SIRT7 is enriched in the nucleolus. However, during M phase when the nucleolus disintegrates, it is associated with chromosomes (8). In addition, the nuclear localization signal (NLS amino acids 61~76, LQGRSRRREGLKRRQE) and nucleolar localization signal (NoLS amino acids 392~400, KRTKRKKVT) have been found in SIRT7 sequence (9). However, the basic residues in either NLS or NoLS are effective in nucleolar localization. Moreover, it has been reported that SIRT7 in young primary fibroblast is detected in both cytosol and nucleolus. However, there is a progressive decline in the level of nucleolar SIRT7 during replicative senescence (9).

REGULATION OF SIRT7 EXPRESSION

In hepatocellular carcinoma, SIRT7 expression is downregulated by miR-125-a-5p and miR-125b (10). In mice, spleen, liver, and testis exhibit high expression of SIRT7 protein, whereas muscle, heart, and brain exhibit low expression (8). However, *SIRT7* mRNA is ubiquitously expressed in various human organs, although the level in spleen is relatively low (4). *SIRT7* transcript levels decrease during aging in mouse hematopoietic stem cells (11), whereas its levels increase during passage of human mammary epithelial cells (12). As mentioned, nucleolar SIRT7 decreases during replicative senescence in normal human lung firbroblasts (9). Based on observations to date, SIRT7 appears to regulate cell proliferation, although its role as a positive or negative factor might differ among organs and cell lineages. In response to oxidative stress, SIRT7 is very slightly downregulated in embryonal rat heart-derived H9c2 cells (13). The regulation of SIRT7 expression remains to be determined in different tissues as well as in contexts such as proliferation, differentiation, and stress responses.

ENZYMATIC ACTIVITY OF SIRT7 DEACETYLASE

The deacetylase activity of SIRT7 has been controversial since the substrate for deacetylation has not been fully revealed. Although p53 interacts with SIRT7 and is deacetylated by SIRT7

in vitro (14), p53 acetylation is not affected by SIRT7 either *in vivo* or in cells (4, 15). Consequently, p53 is not widely accepted as a direct substrate for SIRT7 *in vivo*. However, SIRT7 exhibits affinity for histone H2A/H2B and histone H3 *in vitro*, supporting that SIRT7 is associated with chromatin (8). Indeed, histone H3 Lys 18 is a specific substrate of SIRT7, suggesting that SIRT7 might play a role in transcriptional regulation (15).

The deacetylase activity of SIRT7 is dependent on NAD^+. Therefore, whereas resveratrol activates the SIRT7 deacetylation of p53 peptide *in vitro*, nicotinamide inhibits SIRT7 activity for H3K18 deacetylation (14, 15).

DIVERSE FUNCTIONS OF SIRT7

Physiological function of mouse SIRT7

Vakhrusheva *et al.* demonstrated that homozygous SIRT7-knockout mice die earlier than wild-type mice and exhibit ageing-related phenotypes; by contrast, heterozygous SIRT7-knockout mice do not show any abnormal phenotype (14). SIRT7-knockout mice suffer from kyphosis and lose subcutaneous fat in early life. In addition SIRT7-knockout mice exhibit degenerative cardiac hypertrophy at the age of 7 months, possibly as a result of the activation of the AKT or RAS pathway and increased cytokines. In addition, SIRT7-knockout cardiomyocytes undergo significant apoptosis. The available data indicate that SIRT7 is critical for maintenance of cardiac homeostasis.

SIRT7 functions in the nucleolus

SIRT7 is enriched in nucleolus. In this compartment, SIRT7 is associated with upstream binding factor (UBF) and RNA polymerase I (RNA pol I) and activates transcription of ribosomal DNA (rDNA) by facilitating association of RNA pol I (8, 16). The activation of rDNA transcription is dependent on the catalytic activity of SIRT7 although, as noted above, its specific target has been unknown (8). During mitosis, when rDNA transcription is halted, SIRT7 is phosphorylated by the CDK1-cyclin B complex, and dephosphorylation of SIRT7 may be required for assumption of rDNA transcription (16). In addition, mass spectrometry has confirmed that SIRT7 is a component of a complex containing the B-WICH complex as well as UBF and RNA pol I (17). These findings suggest that SIRT7 facilitates rDNA transcription by interacting with the chromatin remodeling complex B-WICH.

Oncogenic potential of SIRT7

Chromatin immunoprecipitation (ChIP) has revealed that SIRT7 binds promoters that are enriched with H3K18ac, indicating that SIRT7-mediated deacetylation of H3K18 leads to the downregulation of target genes. ChIP-qPCR (quantitative PCR) analysis has shown that SIRT7 regulates the expression of genes related to RNA processing, translation, RNA splicing, and mRNA metabolism. In particular, the tumor-suppressor genes *NME1* and *COPS2* are repressed by the transcription factor ELK4, which targets SIRT7 to these promoters. Thus, SIRT7 is involved in the downregulation of some tumor suppressors. In addition, SIRT7 depletion reduces anchorage-independent growth in cancer cells and E1A oncogene-induced loss of contact inhibition in normal lung fibroblasts (15). Overall, SIRT7 contributes to tumorigenesis through H3K18 deacetylation.

Recent findings regarding the elevated level of SIRT7 in cancers support the idea that SIRT7 possesses oncogenic potential. *SIRT7* mRNA levels are higher in breast cancer tissue than in normal breast tissue, and they are further upregulated in node-positive breast cancers relative to node-negative breast cancers (12). In addition, the levels of both *SIRT7* mRNA and SIRT7 protein are elevated in hepatocellular carcinoma (10). Although an initial report was retracted due to incorrect findings, SIRT7 seems to be increased in thyroid cancer (18). In contrast

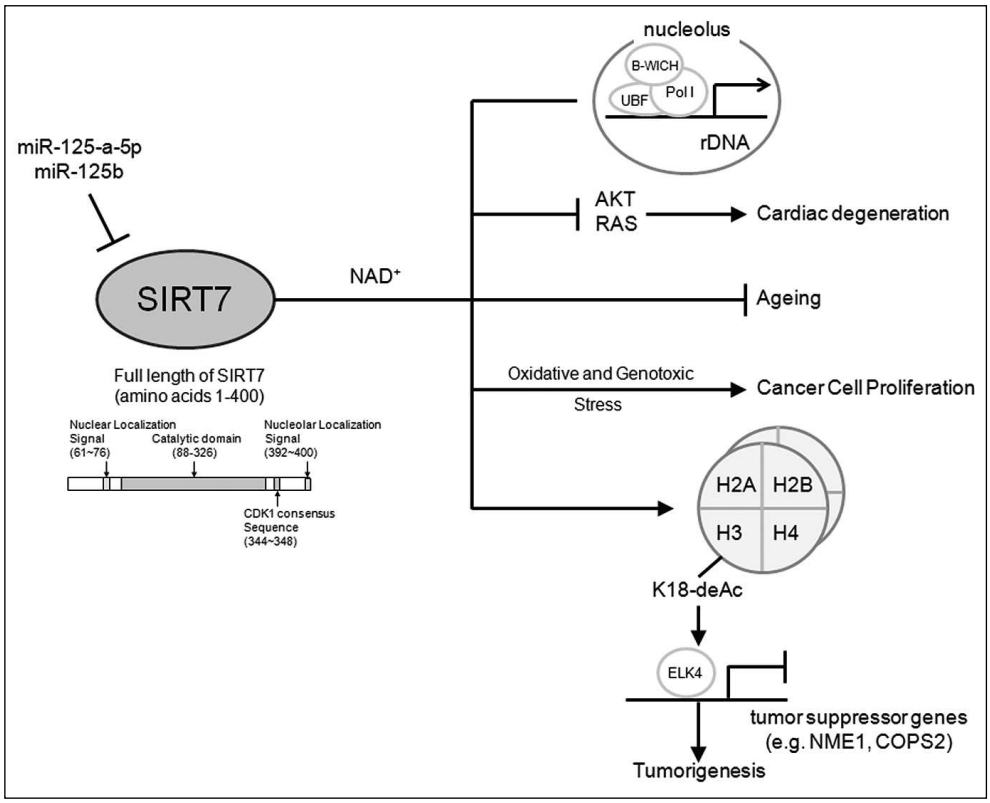

Fig. 1. Diverse functions of NAD^+-dependent SIRT7 deacetylase. SIRT7 enhances rDNA transcription in nucleolus. In addition, SIRT7 is required for cardiac development and homeostasis. In regards to cancer, SIRT7 regulates cancer cell proliferation upon oxidative and genotoxic stress and represses transcription of tumor suppressor gene.

to these findings, *SIRT7* mRNA levels are lower in tumorigenic murine cell lines than non-tumorigenic cell lines (19). *SIRT7* mRNA and SIRT7 protein levels are downregulated in HNSCC (head and neck squamous cell carcinoma) compared to normal tissues, and they are lower at advanced stages of HNSCC than at earlier stages (20). Overall, the link between SIRT7 expression and tumor development is not clear yet. Further investigation will be necessary to determine whether SIRT7 acts as an oncogene in other types of cancers.

Regulatory functions of SIRT7 in survival and apoptosis

Accumulating evidences indicate that SIRT7 participates in the regulation of cell proliferation. SIRT7-overexpressing 10T1/2 mouse embryo fibroblasts exhibit low colony-forming efficiency (19). In contrast to this inhibitory effect on survival in normal cells, SIRT7 is required for survival of cancer cells. Transfection of siRNA targeting SIRT7 reduces the proliferation of hepatocellular carcinoma cells, possibly through G1 cell cycle arrest and autophagy, as well as that of osteocarcinoma cells through apoptosis (8, 10). In addition, SIRT7-deficient hepatocellular carcinoma xenograft tissues also exhibit reduced tumor size, further illustrating the pro-survival function of SIRT7 in cancer cells. SIRT7-deficient cardiomyocytes lose resistance to stress-inducing stimuli such as hydrogen peroxide and adriamycin (14). Overall, these findings suggest that SIRT7 is required for cell survival in the presence of oxidative and genotoxic stress as well as for normal proliferation.

The effect of SIRT7 in hypoxia signaling

SIRT7 interacts with HIF-1α and HIF-2α and then downregulates the expression level of HIF-1α and HIF-2α protein although its degradation is known to be independent of proteasomal or lysosomal pathway. However, it implicates that the SIRT7 inhibition may be critical for the proper hypoxia signaling (21).

CONCLUSION

SIRT7 may play critical roles in the maintenance of homeostasis and cellular metabolism in both normal and cancer cells (*Fig. 1*). However, the detailed mechanisms underlying these functions have not yet been elucidated since the deacetylation substrates of SIRT7 relevant to each function remain unknown. The identification of SIRT7 substrates will improve our understanding of the functions and mechanisms of SIRT7 deacetylase. In addition, because SIRT7 may attenuate ageing and promote tumorigenesis, the development of specific activators or inhibitors will make it possible to widen the therapeutic windows for controlling ageing and curing diseases such as cancer. Unlike SIRT1 or SIRT2, for which many specific activators or inhibitors have been developed, there are currently no specific small molecule activators or inhibitors of SIRT7. The development of proper therapeutic drugs would contribute to inhibit tumorigenesis through the apoptosis of cancer cells, inhibition of epithelial-to-mesenchymal transition (EMT) and anti-angiogenesis (22-24). Therefore, further efforts to identify and develop SIRT7-targeting drugs will increase the utility of SIRT7 as a therapeutic target.

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